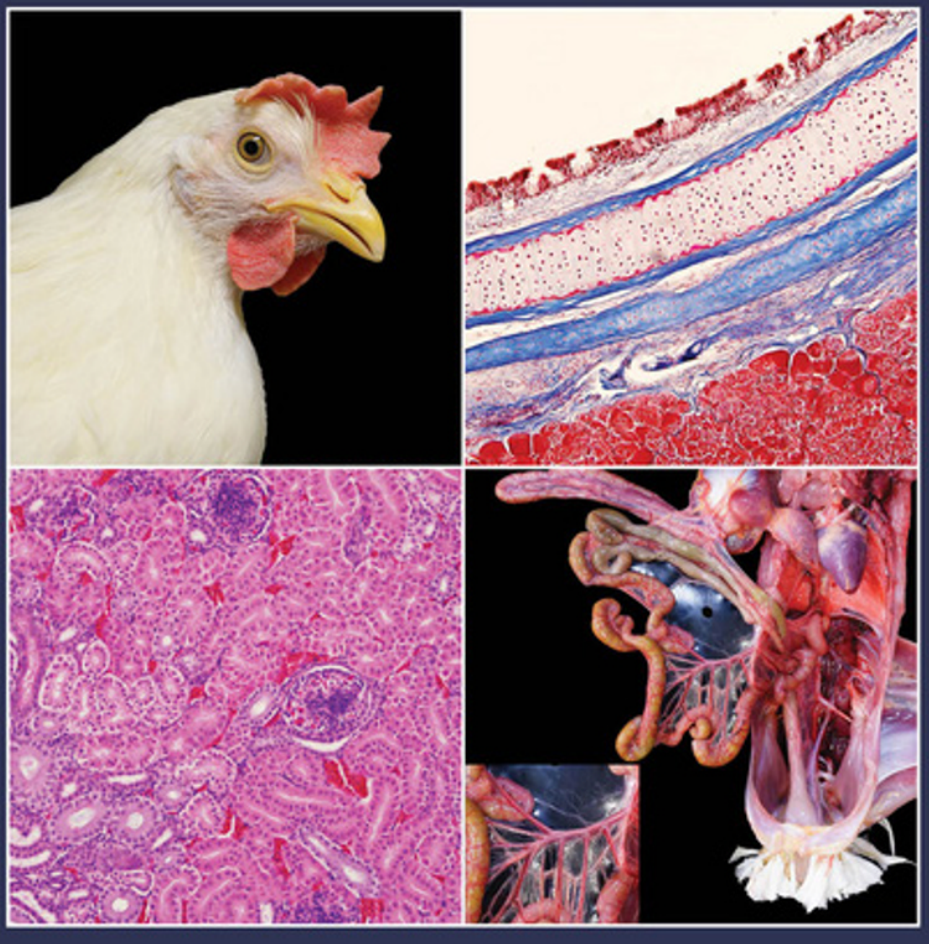
Anatomy and Histology of the Domestic Chicken

Edited by **Wael Khamas** • **Josep Rutllant**



WILEY Blackwell

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Dedication

To My beloved family. I dedicate this book to you with profound gratitude and a heart full of love. Your support, patience, and endless encouragement have been the guide on this journey. May this book be a token of my appreciation and a reminder of the unbreakable bonds we share.

Wael Khamas

This book is dedicated to my parents, who have consistently instilled in me the treasured values of learning, diligent work, integrity, civility, and the significance of friendship. "Mai us podré tornar tot el que heu fet per mi" – I can never fully repay you for all that you've done for me. To Helena and Laura, for the boundless love and unwavering support you have graciously bestowed upon me. Lastly, to Joaquín Camón, a cherished mentor who introduced me to the world of anatomy and firmly believed in my potential. You are all notes of my music.

Josep Rutllant

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Foreword

The chicken...one of the most unassuming animals in the world yet its importance in agricultural food production cannot be underestimated. As a food source, chicken is the leading protein source for most of the global population. Chickens have been recommended for farming in developing countries to supply both meat and eggs. In addition, the chicken has been increasing in popularity among individual families to have them roaming in backyards for insect control, nostalgia, and food sustainability. This was most evident during the global COVID-19 pandemic where families sought to have their own egg source for their families. In addition, the chicken has become more of a family pet so ensuring their health and welfare is also important from a veterinary medical standpoint. Unfortunately, there is a scarcity of literary resources that one can consult when working with the chicken.

Having spent my entire career working in poultry and avian medicine, I know that there is a lack of resources that are readily available. It is with great anticipation that I await the Anatomy and Histology of the Domestic Chicken. Drs. Wael Khamas and Josep Rutllant-Labeaga have assembled a team of anatomists and veterinarians to create a most comprehensive 16-chapter reference of the chicken anatomy and histology. The authors document all areas of the chicken starting from the external anatomy and going through each organ system, including the special senses. In addition, the authors cover a much-needed area, which is the avian egg along with fertilization and chicken development. Never before has there been one comprehensive source on the chicken. Moreover, in addition to covering all major body parts of the chicken, the authors were very insightful to also include related clinical anatomy of the chicken, including the necropsy of the chicken which would be very useful for diagnostics and clinical poultry medicine. Moreover, the book is composed of many visually stunning photographs, including the internal anatomy of the cloaca. Many references in the past used schematic diagrams, but this is a masterpiece for readers.

This book will serve as a resource for so many individuals, from researchers to educators. At the undergraduate and graduate level, I envision this book serving as a resource for students learning zoology and biology in comparative anatomy courses; and for animal science courses, where students can learn about the chicken, a major species in agricultural production. This book will also be of interest to evolutionary biologists and paleontologists since the chicken is a descendant of the dinosaurs, and diseases of the chicken have been used as a model for the potential diseases that may have affected dinosaurs. At the professional education levels, this book will be very valuable for those studying veterinary medicine and learning about the chicken as the chicken is also the model for other avian species. This book will serve as a resource for those veterinarians and scientists working with the chicken. Hence, it is with great anticipation that I look forward to having this book in my library and adding it to my collection of chicken-related books. Not only does it provide resources in chicken anatomy and histology, but the book is composed of stunning photography for the chicken aficionado and hobbyist. There is no doubt that this book will be a timeless, valuable global resource for the chicken.

Teresa Y. Morishita, DVM. PhD. Dipl. ACPV

Preface

The domestic chicken, *Gallus gallus domesticus*, has played an integral role in human history and sustenance for thousands of years. From its origins as wild junglefowl to becoming one of the most widespread and versatile domesticated animals today, chickens have captured the imagination and attention of researchers, breeders, veterinarians, and enthusiasts alike.

This book, "***Anatomy and Histology of the Domestic Chicken,***" is a comprehensive and meticulously crafted resource that delves into the intricacies of the avian body. Addressing a diverse audience, including veterinary professionals, veterinary students, animal science students, poultry researchers, chicken breeders, and backyard chicken keepers, this work aims to provide a deep understanding of the anatomy, histology, and physiological systems of domestic chicken.

In sixteen meticulously organized chapters, this book covers all major body systems, including the musculoskeletal, respiratory, cardiovascular, digestive, urogenital, and nervous systems. Each chapter is structured to provide a comprehensive overview of the relevant anatomy and histology, coupled with insights into the physiological functions specific to chickens. Additionally, special emphasis has been placed on clinical applications, as understanding the anatomical and histological basis is essential for diagnosing and treating poultry diseases and disorders.

The journey through the avian body does not stop with the mature bird. This book delves into the miraculous world of the egg and chick embryonic development, exploring the fascinating aspects of fertilization and embryology. Understanding the intricate processes of embryonic growth can significantly impact the poultry industry and backyard enthusiasts, optimizing breeding practices, and enhancing the well-being of these feathered companions.

As our understanding of avian biology continues to evolve, this book seeks to provide readers with the most up-to-date information available up to its publication date. We hope that this work serves as a valuable reference, both in the academic realm and practical applications, assisting veterinarians in diagnosing and treating poultry ailments, researchers in pushing the boundaries of avian science, and chicken enthusiasts in raising healthy and thriving flocks.

The development of this book would not have been possible without the contributions of numerous experts in the fields of veterinary anatomy and medicine, and poultry science. Their passion for advancing knowledge in avian anatomy and histology has enriched the contents of this volume.

As we embark on this journey through the anatomy and histology of the domestic chicken, we invite our readers to explore the wonders of avian life, marvel at the intricacies of their anatomy, and embrace the opportunities to enhance the well-being of these remarkable creatures.

We hope that this book serves as an indispensable guide for all those interested in the fascinating world of domestic chicken, contributing to the advancement of poultry science and the welfare of these cherished birds.

Acknowledgments

We would like to express our gratitude to numerous individuals who have played a vital role in the preparation of this book. We owe a debt of thanks to our colleagues, students, and technical staff, all of whom have contributed significantly to this endeavor.

Our veterinary medicine students have been instrumental in shaping the content of this book. Their inquisitive nature and thoughtful questions during laboratory sessions, particularly in bird cases, encouraged us to delve deeper into the subject matter. We carefully considered their queries while planning each chapter, ensuring that we addressed their concerns.

A special acknowledgment goes to Dr. Teresa Morishita, whose belief in our capabilities led her to involve us in writing two separate chapters: one on Backyard Poultry Medicine and Surgery and another on Gamebird Medicine and Management. Her unwavering support and encouragement were instrumental in our commitment to completing this book.

We are also grateful to the technical staff, led by Kevin Mondragon and his team, at the College of Veterinary Medicine, Western University of Health Sciences. Their expertise in specimen preservation, skeleton preparation, and camera settings greatly facilitated our work, especially during the photography sessions. Their dedication deserves our sincere appreciation.

We extend our heartfelt thanks to all the chapter writers who generously shared their expertise by contributing to this book. Your willingness to share your knowledge is truly commendable.

Dr. Alexandra Gean played a pivotal role in enhancing the readability of several chapters. Her valuable input and organization of paragraphs ensured a smooth transition of information from one topic to the next. Her support and encouragement to this project were contagious. Thanks Ally.

We extend our heartfelt gratitude to Dr. Miguel Saggese for his generous and invaluable contributions to the review of chapters for this book. His expertise and meticulous attention to detail have greatly enriched the quality of our work. We are profoundly thankful for his commitment and collaborative spirit, which have made a significant difference in the success of this book.

We would like to acknowledge the contributions of Wiley's collaborators, who provided invaluable insights that guided our writing process, particularly in the initial chapters. Your assistance saved us time and kept us on track.

Finally, we are grateful to Western University of Health Sciences for their support, financial and logistic. Their continuous encouragement has been instrumental in our efforts to complete this book and make it accessible to all who wish to learn from it.

Lists of Abbreviations

Chapter 1
None

Chapter 2
None

Chapter 3

ATP	adenosine triphosphate
M	muscle

Chapter 4

cm	centimeter
SEM	scanning electron microscope.

Chapter 5

mm	millimeter
μm	micrometer

Chapter 6

JG	juxtaglomerular
MD	macula densa
PAS	periodic acid-Schiff

Chapter 7

SST	sperm storage tubules
UVJ	utero-vaginal junction
LMSP	male sperm precedence
LH	luteinizing hormone
OIH	ovulatory-inducing hormone
GnRH	gonadotropin-releasing hormone
ZPs	zona pellucida glycoproteins
AR	acrosome reaction
PTX	pertussis toxin

Chapter 8

GH	growth hormone
HPA	hypothalamic-pituitary-adrenal
PLP	prolactin-like protein
IGF1	insulin-like growth factor-1
PVN	paraventricular nucleus
POA	preoptic area
ARC	arcuate nucleus
PVN	Paraventricular nucleus
SCN	Suprachiasmatic nucleus
TRH	thyrotropin-releasing hormone
CRH	corticotropin-releasing hormone
GnRH	gonadotropin-releasing hormone
FSH	follicle-stimulating hormone
LH	luteinizing hormone
ACTH	adrenocorticotropic hormone
PRL	prolactin
TSH	prolactin, thyroid-stimulating
ADH	antidiuretic hormone
MSH	melanocyte-stimulating hormone
T4	thyroxine
T3	triiodothyronine
PTH	parathyroid hormone
UB	ultimobranchial bodies

Chapter 9

mm	millimeter
HZ	hertz

Chapter 10

AV	atrioventricular node
a	artery
H. & E.	hematoxylin and eosin

Chapter 11

MALT	mucosal associated lymphoid tissues
GALT	gut-associated lymphoid tissues
Ab	antibody
Ig	immunoglobulin
mRNA	messenger ribonucleic acid
Ig	immunoglobulin
M cell	microfold cell
MHC	major histocompatibility complex
PALS	periarterial sheaths
PELS	peri-ellipsoidal lymphocytes sheaths
CALT	conjunctival associated lymphoid tissue
MALT	mucosal-associated lymphoid tissue
BALT	bronchiole-associated lymphoid tissue

Chapter 12

CNS	central nervous system
PNS	peripheral nervous system
CSF	cerebrospinal fluid
L2	lumbar 2
L3	lumbar 3
L4	lumbar 4
S1	sacral 1
S2	sacral 2

Chapter 13

PCR	polymerase chain reaction
gm	gram

Chapter 14

LED	a light-emitting diode
spp	species

Chapter 15

None

Chapter 16

SST	sperm storage tubules
UVJ	utero-vaginal junction
LMSP	last male sperm precedence
OIH	ovulatory-inducing hormone
ZP	zona pellucida
AR	acrosome reaction
PTX	pertussis toxin

1

External Features of Chicken

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1.1 Skin

The chicken skin is thin, loosely attached to the hypodermis, and in most bird species, it has pale pink or bluish pink color. In some species, it can be yellow and even blackish colored based on the presence of xanthin, carotenes, and melanocytes. When compared to mammals, the avian skin is, for most species, dry, often translucent, and inelastic over most of the body, which makes it prone to tears (Nett and Tully 2003). As an additional adaptation for flying, the bird loosely fits inside its skin over most of the body. In some regions, it is strongly attached to the underlying tissue with little or no modification with a very small dermis, like in the skull and the wing tips.

The integument is highly modified on the beak, feet, and certain parts of the bird body such as the ornaments (wattles and crest). The avian skin is the largest organ and acts as the first line of defense against pathogens. It is also involved in heat regulation and prevents the loss of body fluids. It is the largest sensory organ in the body, having receptors for temperature, pain, pressure, and tactile discriminations (Lucas and Stettenheim 1972, p. 1975, pp. 485–486).

The skin has two main types of keratins: (i) soft keratin, which is present in the body and ornaments (alpha keratin) and (ii) hard keratin, found on the scales, spurs, beak, and feathers (beta keratin) (Greenwold et al. 2014). Both male and female may develop thickened areas within the dermis on the ventral abdominal region corresponding with the brooding or incubation patches.

The nomenclature used in this chapter are mostly from Nomina Anatomica Avium (Baumel et al. 1993).

Most of the skin is covered with feathers and no glands are present except for the aural, third eyelid, vent, and uropygial regions. The absence of sweat glands indicates that birds thermoregulate by panting and gular fluttering, among other mechanisms and behaviors. The thick coat of feathers limits the exchange of heat with the environment, a significant difference with mammal's skin. In certain regions of the chicken body, the epidermal cells of the skin produce a holocrine lipidic secretion, such as in the rictus, interdigital web, and in the uropygial gland (Menon et al. 1981).

The chicken skin is divided into the external epidermis and the deeper dermis that is anchored to the underlying hypodermis.

1.1.1 Epidermis

The epidermis is thin in feathered regions and thick in bare regions. The epidermis is composed of stratified squamous epithelium; the number of layers varies depending on the body region. The epidermis, except in the comb and wattle, is mostly composed of different strata (singular stratum): germinativum or basale, spinosum, granulosum, lucidum, and corneum. Not all layers/strata described in mammals are present in the epidermis of the thin skin of the chicken like the stratum lucidum. The avian epidermis is composed of one to six layers of epithelial cells depending on the body region. This includes the basal (germinal) layer, composed of simple cuboidal cells, an intermediate section of several layers composed of cuboidal cells (stratum spinosum), and the stratum corneum, composed of flattened cells without nuclei. The stratum corneum varies in thickness from region to region (Figure 1.1A and B).

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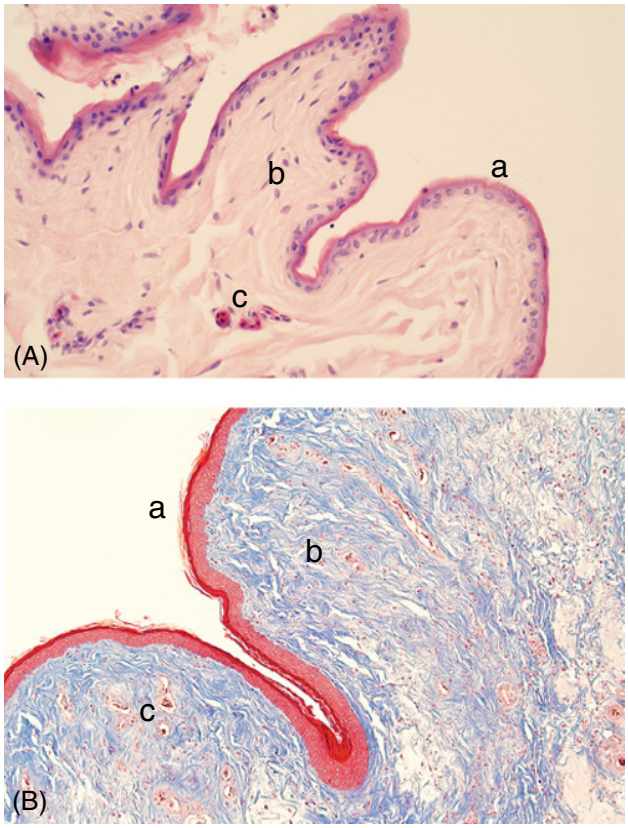


Figure 1.1 (A and B) Histology sections of abdominal chicken skin. This extremely thin skin layer shows (a) slightly keratinized stratified squamous epithelium, (b) dermis, (c) blood vessels. (A) H&E and (B) trichrome stains.

1.1.2 Dermis

The dermis consists of irregular connective tissue of variable thickness between the subepithelial region to the deep dermis close to the hypodermal layer and adipose tissue. Three layers can be identified, the *stratum superficialis* being the more superficial, and consisting of irregular connective tissue with low innervation and in close contact with the *stratum basale* of the epidermis. A deeper layer, the *stratum profundum*, on the contrary, is richly innervated, and can be subdivided into two distinct regions or sublayers, the *stratum compactum* and the *stratum laxum*. The *stratum compactum* by name is a thick irregular connective tissue rich in collagen and with a small number of elastic fibers. This layer is filled with blood vessels of variable sizes and with bundles of nerve fibers. The *stratum laxum* consists of loosely arranged connective tissue, smooth muscle, and abundant adipose tissue. Homberger and Silva (2000) reported that the fat deposits in the *stratum compactum* and *stratum laxum* of the avian skin act as a noncompressible hydraulic tissue for the movement of feathers inside the dermis. This region mostly harbors contour feathers which are seen surrounded by a layer of collagen fibers (Bharathi et al. 2018).

1.1.3 Hypodermis (Subcutis)

The hypodermis, *subcutis*, or subcutaneous layer underlays the dermis, but it is not considered part of the skin. It consists of a layer of connective tissue rich in collagen bundles but with few elastic fibers. It is highly vascularized, innervated, and has lymphatic drainage. In gross anatomy, this layer is the superficial fascia and can be divided into three different layers: (i) the superficial layer, which is immediately below the dermis, (ii) a middle layer that may have adipocytes and called panniculus adiposus in mammals which extends in between skeletal muscles (intermuscular fascia), and (iii) the deeper layer or fascia, located between the skeletal muscles and the bone, and blending with the periosteum.

1.1.4 Blood Supply of the Skin and Nerve Ending

The blood supply to the chicken skin derives from larger blood vessels present in the hypodermis that branch into smaller arteries and arterioles when they penetrate the dermis. Blood capillaries branch from them to reach the subepithelial region, directly supplying blood to the upper portion of the dermis and the epithelium through diffusion. Certain regions of the skin and its associated structures, such as the ornaments, comb, wattle, and ear lobes, are highly vascularized.

Arteries and veins are usually accompanied by nerve fibers. Mechanoreceptors in the skin correspond to the mammalian Vater Pacini (Pacinian) corpuscle, called Herbst corpuscles in birds. They are present in the feathered skin, beak, and distal leg (Hodges 1974, pp. 11–12). The small type Herbst corpuscles are usually superficially located closer to the feather bulbs while the larger ones are localized deep in the dermis. These encapsulated structures are composed of an inner bulb surrounded by several layers of loose connective tissue. Another corpuscle, described by Gottschaldt (1985) is the Grandry corpuscle, equivalent to the Meissner corpuscles in mammals, present in the beak skin of geese and ducks that respond rapidly to active movement in aquatic environments.

1.2 Structures Associated with the Skin

1.2.1 Patagium (pl. patagia)

The patagia are thin, feathered membranous folds of skin that connect the humeral bone and shoulder with the distal ulna/radius and the carpus that sits along the cranial

border of the wing. The cervical extension of the patagium (referred to as parapatagium), present in front of the shoulder joint which is not part of the wing. The patagium is composed of four parts (prepatagium, metapatagium, postpatagium and alular patagium). The main patagium of the wing is the prepatagium (wing web), between the shoulder and the carpus. This prepatagium is rich in elastic connective tissue which enables the chicken to hold the wings close to the body without muscular effort. The free prepatagium is a triangular fold or thin double fold of skin laying between the radius and ulna and the humerus. The thin free edge of the prepatagium is directed anteriorly (Lucas and Stettenheim 1972, p. 57). The metapatagium is also triangular, a very small fold of skin located between the thorax and the wing. Its edge attached to the wing along the caudal border of the triceps humeralis muscle. The postpatagium is a chevron-shaped skin web that lies caudal to the forewing and the carpal/metacarpal bones, extends from the elbow to the longest digit, and bears the primary and the secondary remiges. The alular patagium is a small web that lies between the adductor indicis muscle (Hudson and Lanzillotti 1972, p. 43) and the tendon to digit III. Its free edge directed toward the tip of the wing (Lucas and Stettenheim 1972, p. 57).

1.2.2 Operculum

The operculum is a cover or a lid present dorsal to the anterior nares (nasal openings) of a bird at the base of the beak (Figure 1.2). The shape and structure may vary from one breed to another.

1.2.3 Ornaments

1.2.3.1 Comb

The most distinctive skin protuberance in the head of the chicken is the comb. Its shape varies among different breeds of chicken. It could be a single crested, rose, pea shaped, or strawberry type of comb depending on the breed. Rooster's combs are always bigger than that of a hen. In poultry production, the comb's size and color are used as features to determine the health and egg production status of the hens. The comb size is related to androgen levels in the male, and this may be associated with the degree of aggressiveness and dominance behavior in both male and female (Candyland 1969). One of the main functions of the comb is to assist in thermoregulation by absorbing light and contributes to the social communication and structure of the flock. The comb is an essential feature to help identify individual animals. Color breed variations reflect also on the color of chicken comb. Fölsch et al. (1994) compared the crest size of Hisex white hens with Hisex brown hens and observed larger and paler combs in white hens compared with brown hens. They stated that this tendency is clearer under artificial light conditions, less light, and higher temperature of the environment in chickens housed in battery cage systems. The comb epidermis has a thin keratinized stratified squamous epithelium. A single basal cuboidal layer (*stratum basale*) and two to three intermediate layers (*stratum intermedium*) correspond to the mammalian spinosum layer in young birds that increase in number in adult birds. The superficial layer is flattened squamous epithelial cells located below the *stratum corneum*. The dermis has a *stratum superficialis*, which is an irregular connective tissue highly vascularized,

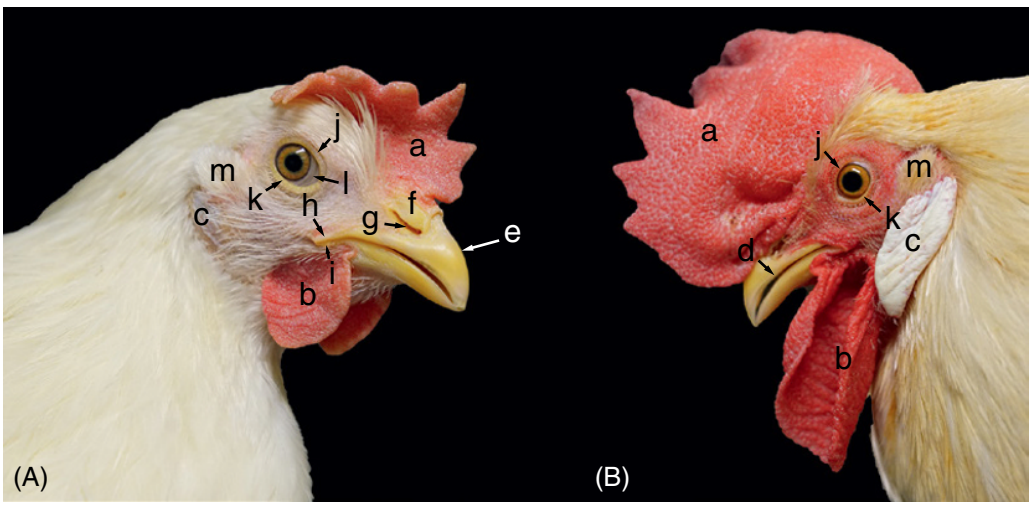


Figure 1.2 Female (A) and male (B) head external features. (a) Single comb, (b) wattle, (c) ear lobe, (d) tomium, (e) culmen, (f) operculum, (g) external nares (nasal opening), (h) maxillary rictus, (i) mandibular rictus, (j) superior eyelid, (k) inferior eyelid, (l) third eyelid (nictitating membrane), and (m) aural feathers.

with arterioles, venules, and capillaries situated close to the *stratum basale*. The *stratum profundum* also has two sublayers: the *stratum compactum* and the *stratum laxum*. The *stratum compactum* is composed of thick, irregular connective tissue rich with collagen but with little elastic fibers. This stratum is filled with medium and small muscular arteries, arterioles, and capillaries, accompanied by bundles of nerve fibers. The glomera of arterioles (a group of arterioles branching and anastomosing with epithelioid cells) are present at the junction of the *stratum compactum* with *the stratum laxum*. The presence of these arterioles acts to reduce the need for heat production when oxygen is low (O'Dea 1990). Therefore, keeping the comb warm does not put burden on the blood circulation. The *stratum laxum* separates the skin from both the hypodermis and the skeletal muscles. It has less connective tissue fibers with larger blood vessels embedded within white adipose tissue. Chicken comb is reported to be rich in hyaluronic acid, which can be used in human medicine for joint lubrication (Almond 2007) (Figures 1.2 and 1.3).

1.2.3.2 Wattle

The wattles are an elongated extension or flap of featherless skin below the head and on both sides of the head. The wattle size and shape vary from breed to breed as the comb does. In general, it is larger in males compared to females, becoming even larger in older males. It is highly vascularized, which results in typical red coloration of the comb. Both wattle and comb become congested during mating season because of the high vascularity in the subcutis which results in increasing the rigidity and in the intense color of these structures (Nickel et al. 1977, p. 159). The skin of the wattle is composed of epidermis and dermis. The epidermis is thin keratinized stratified squamous epithelium of five to six cell layers. The basal layer (*stratum basale*) is simple columnar followed by two to three cuboidal layers of middle layer (stratum intermedium). The outermost layer is simple squamous flattened cells below the thin keratinized layer (stratum corneum). The dermis has a more superficial layer which is highly vascularized (arterioles, venules, and capillaries) close to the *stratum basale* and a thinner connective tissue with blood vessels and nerve fibers. The dermis of the wattle is a thin loose connective tissue filled with blood vessels and nerve fibers (Figures 1.2 and 1.4).

1.2.3.3 Ear Lobes

The earlobes are featherless skin extending down below the external ear openings. It is usually red in color because of the presence of extensive blood supply (capillaries and venous sinuses) which are present immediately under the epidermis (Figure 1.2). Though, in certain breeds, they

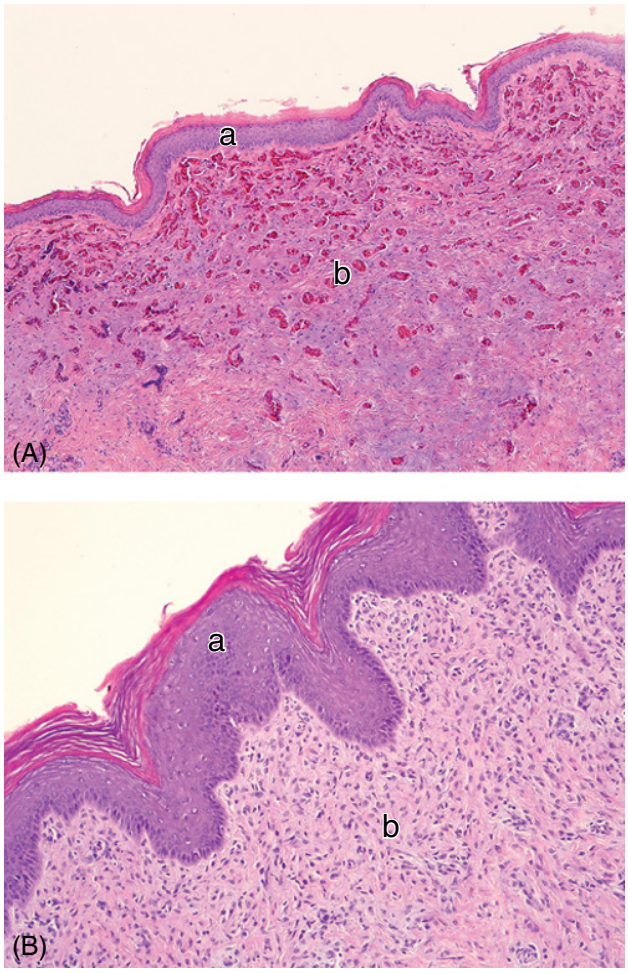


Figure 1.3 Comb of young (A) and adult rooster (B) showing keratinized stratified squamous epithelium with higher number of stratifications of cells within the epidermis (a), dense irregular connective tissue of the dermis (b). H&E stain.

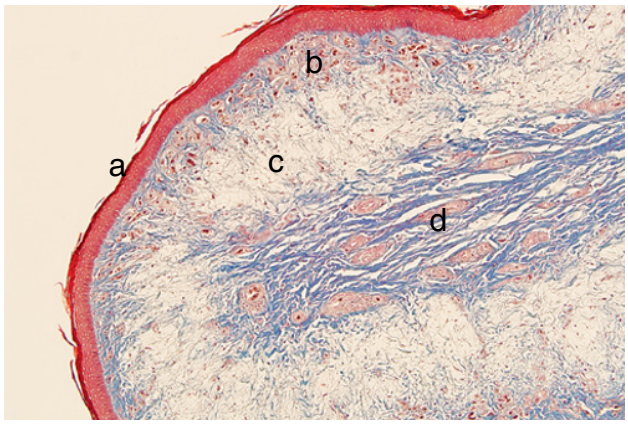


Figure 1.4 Wattle of a rooster showing thin keratinized stratified squamous epithelium (a), highly vascularized upper layer of the dermis (b), thin loose connective tissue from middle layer of the dermis (c), and deeper layer of the dermis (d). Trichrome stain.

could be white. The color of the earlobe usually is correlated with the color of eggs in laying hens. Chickens with red earlobes tend to lay brown eggs while those with white earlobes tend to lay white eggs (Morishita et al. 2021, p. 464). The ear lobe is covered by keratinized stratified squamous epithelium of five to six layers total thickness. These cells are two to three cuboidal cell layers and two to three flatted squamous superficial cells. The dermis forms a highly vascularized dense irregular connective tissue below the epithelium. Deeper, the dermis is less dense than the superficial layer with the presence of elastic fibers and large blood vessels. Resident macrophages (histiocytes) are present in addition to the regularly present fibroblast/fibrocyte. Elastic fibers and variable amount of connective tissue density were observed by the authors depending on the sectioned region of the ear lobe. The epithelial layer thickness of the ear lobe increases along the attachment side to the head. Herbst corpuscle close to the feather's follicle is small, while deeper corpuscles are larger.

1.2.3.4 External Ear Opening

The external ear canal opening of the chicken is caudal to the quadrate bone. Auricular glands are present on the ventral wall of the canal. These glands open into a duct and can be visualized under higher magnification (König et al. 2016, p. 243). Aural sebaceous glands around the external ear secrete a waxy substance (Menon and Salinukul 1989). Six classes of neutral lipids are identified by thin layer chromatography of the lipid materials extracted separately from the secretion as well as from the isolated whole glands present in the skin of the floor of the external ear canal of the domestic fowl (Dutta et al. 1997).

1.3 Feathers

Feathers are important structures and perform several functions during the bird lifetime including flight, thermoregulation, water repellent, communication, water transport, transfer of plant seeds, sound production, and brooding (incubation of eggs) (Lucas and Stettenheim 1972, pp. 257, 276).

1.3.1 Development

The somatopleure (ectoderm and somatic mesoderm) expansion and closure leads to the juxtaposition of the ventral pteryla. The embryonic proximal somatopleural mesoderm is destined to form a feather-forming dermis at two days of incubation (Fliniaux et al. 2004). Other researchers stated that early development of the feathers from pterylae start at day six of incubation of having a dermal pulp (sheath and basal layers) (Prum and Dyke 2003). Epidermal placode elongates forming a short bud with a tubular central dermal tissue. The epidermis forms the barb ridges and barbs of the first natal down. The pulp starts from the germ cells to produce the entire feather. Feathers are replaced/renewed throughout the entire life of the bird in a sequence of (continue for most species) events called molt. Feathers are entirely epidermal in origin and lined with epidermal cells which are anchored inside the dermis after passing through the epidermis and sometimes the hypodermis. Prum and Brush (2002) stated that a feather follicle differs from a hair follicle in that the follicular invagination is not merely a depression in the epidermis but a circular trough that encircles a persistent dermal papilla. Both primary and secondary feathers attach close to the periosteal layer of the bones.

1.3.2 Description of Feathers

Feathers are skin appendages like the glands of mammalian skin. They are tubular and widely variable in shape and chemical composition of the arrangement and numbers of keratinocytes (Prum and Dyke 2003) depending on their location and/or function. Morphologically, feathers are considered homologous of reptilian scales. However, in development, morphogenesis, gene structure, protein shape, sequence, filament formation and structure, feathers are different from scales (Brush 1996).

Researchers have noted the role of feathers in repelling parasites alone or in conjunction to earth elements. In a study conducted by Martin and Mullens (2012), they permitted chickens infested with lice to apply sand, litter, or kaolin (a fine clay) to their feathers for dusting. The use of kaolin resulted in significant reductions in lice populations, whereas dusting with sand or litter had little to no effect. Likewise, Vezzoli et al. (2015) reported that dusting with sand showed no impact on ectoparasitic mites.

1.3.3 Ptilosis and Pterylosis

Ptilosis refers to the complete set of feathers (plumage) associated with the feather's follicles, while pterylosis is the arrangement of feathers within certain tracts over the bird skin. On one plucked adult rooster (the authors prepared), different tracts including ventral and dorsal views were labeled (Figure 1.5A–C). The term pteria is a part of the bird skin where feathers grow, while apteria is the naked spaces between feathered skin, for example, on both sides of the chicken neck. Detailed feather tracts (pterylosis) of the chicken are described by Lucas and Stettenheim 1972, pp. 74–75).

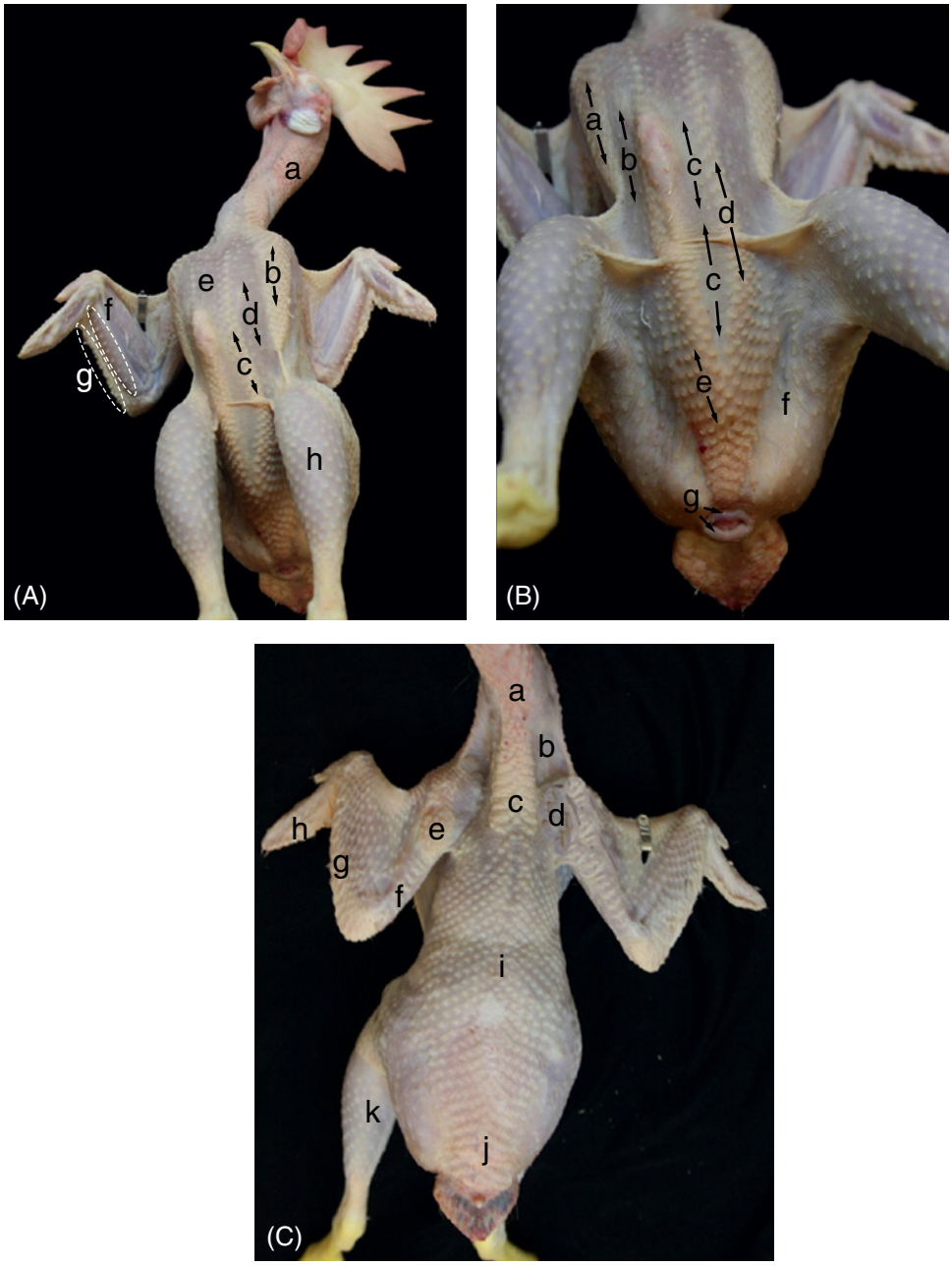


Figure 1.5 (A) Ventral view of a plucked adult rooster. (a) Lateral cervical tract, (b) pectoral tract, (c) sternal tract, (d) pectoral apterium, (e) sternal apterium, (f) secondary coverts, (g) secondary feathers, and (h) crural tract. (B) Ventral view of plucked adult male chicken. (a) Pectoral tract, (b) pectoral apterium, (c) sternal apterium, (d) sternal tract, (e) abdominal tract, (f) abdominal apterium, and (g) anal circulet. (C) Dorsal view of a plucked adult male chicken. (a) Dorsal cervical tract, (b) lateral cervical apterium, (c) interscapular tract, (d) scapular apterium, (e) humeral tract, (f) post humeral tract, (g) upper secondary feathers, (h) primary feathers, (i) dorsopelvic tract, (j) dorsal caudal tract, and (k) crural tract.

1.3.4 Feathers Color

Feathers can be of any color or mix of colors. However, color, structure, and shape may vary after molting. Melanin is a common pigment in chicken skin. Melanin is produced by melanocytes (a neural crest cell in origin). These cells are situated at the basement membrane of the skin and have pseudopodia to extend between the keratinocytes (all epidermal cells) of the epidermis. Through the process of transcellular movements, the melanosomes (melanin pigment within a single layer membrane) are phagocytosed by the keratinocytes (Sharlow et al. 2000). The second most common pigment is the carotenoid, a large group of

pigments produced by plants and other organisms. Once absorbed, carotenoids are transported in fat globules through the blood to the dermal feather pulp where they are selectively absorbed by the keratinocytes (Brush 1978). Red coloration comes from carotenoids, yellow from either carotenoid or melanin, while pink is a mixture of both red and yellow. If melanin pigments reach the epidermis in substantial amounts, these black pigments result in darkening the entire skin of the chicken, like in Silkie chickens. Green color comes from the lipochrome in the epidermis and melanin in the dermis. Other colors result from the combination of different pigments and physical properties of the feathers (Getty 1975, pp. 2071–2081).

1.3.5 Types of Feathers

1) Natal down feathers refer to the layer of early down feathers that cover the bird at a certain stage of early development. Birds can be classified as altricial or precocial. Chicken is a precocial bird that is covered with natal down after hatching. The hatchlings of altricial birds are almost naked. This divergence is thought to reflect environmental adaptation, but the molecular basis of the divergence is unclear (Chen et al. 2016).
2) Blood feather is the one that is growing and or replacing the shed feather. The feather needs lots of nutrients to continue to grow and that is why blood filled the tubular portion of the feather which reflects the name. The blood vessels supplying this feather regress once the feather is fully developed.
3) Filoplume is a hair-like (filum-thread) that remains on the skin when all other feathers are removed. Filoplumes are present in all feather tracts of the body. There are usually more than two for each remix (flying feather) or rectrix (tail feather). They are associated with contour feathers and may be sensory or decorative in function.
4) Alular feathers are a specialized structure of the upper leading edge of a bird's wing that consists of a tuft of short flight feathers usually three in number attached to the movable first wing digit corresponding to the thumb to facilitates flight, landing, and maneuverability at slow speeds (Figure 1.6). By altering airflow, the alulae permit good maneuverability and control at low flying speeds which are crucial for takeoffs and landings (Linehan and Mohseni 2020).
5) Down feathers in adult birds are a layer of fine feathers under the main coat of the bird feathers.
6) Rictus is the soft tissue border of the mouth made of skin from the commissure angle forward into the proximal mandible and maxilla (Figure 1.2).

Figure 1.6 Rooster alular feathers (short flight feathers, usually three) normally attached to the movable first wing digit to facilitate flight, landing, and maneuverability at slow speeds.

7) Flying feathers include the primary, secondary, and tail feathers. The flying feathers consist of the shaft (rachis and calamus), barbs, and barbules.
8) Contour feathers are the predominant type of feathers, and they are larger in number compared to flying feathers. Length and shape of contour feathers varies from region to region (Lucas and Stettenheim 1972, p. 235).
9) Auricular feathers are modified contour feathers. They form several rows around the ear opening. They are of two types, rostral and caudal coverts that are arranged in a way to not hinder sound passage but prevent passage of insects and foreign materials (Getty 1975, pp. 2027 and 2082).
10) Bristles feathers in chicken are present only in the cilia of the eyelids (eyelashes).
11) Powder feathers are absent in chicken but present in pigeons.

1.3.6 Parts of Feather

Rachis: The rachis is the long solid portion of the feather shaft above the skin. The rachis carries the vanes composed of barbs connected by barbules in an interlacing pattern (Hodges 1974, p. 15) (Figure 1.7).

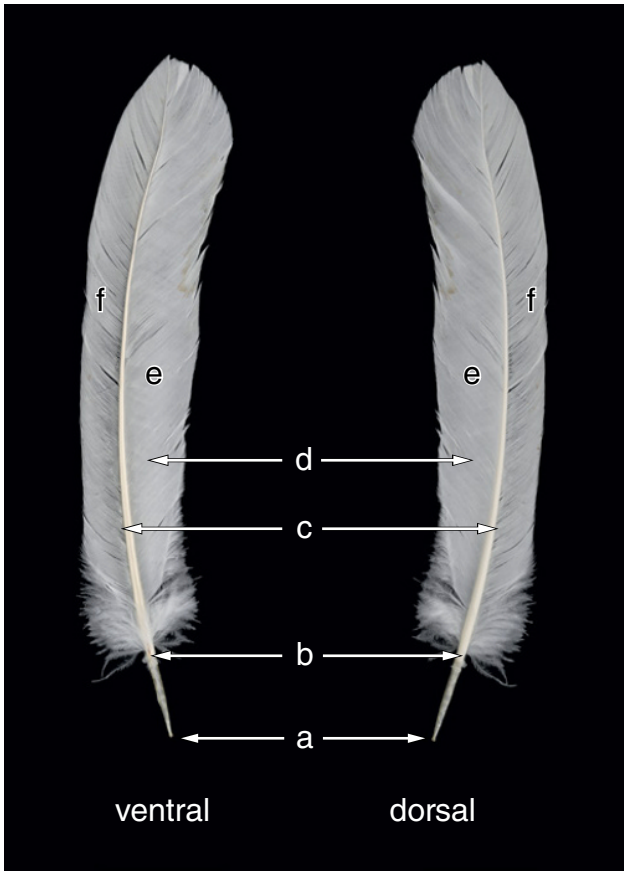


Figure 1.7 Adult feather from the tail. (a) Inferior umbilicus, (b) calamus (quill), (c) rachis, and (d) barbs. If this was a primary feather from the wing, (e) it would be considered posterior barbs and (f) anterior barbs.

Calamus (quill): The calamus is the hollow inner portion of the feather shaft that lacks barbs and attaches to the skin. The calamus is the bare portion of the feather and partly lies within the feather follicle (Lucas and Stettenheim 1972, p. 235). It is the tubular structure of the feather composed of non-pigmented stratified squamous epithelium and has transverse partitions within the cavity. It is separated from the rachis by the superior umbilicus opening.

Umbilicus: Inferior (distal) umbilicus contains the dermal papilla at the end of the feather inside the dermis while the superior (proximal) umbilicus always presents at the neck of the follicle where the dermal papilla projects into it.

Barb (ramus Pl. rami): *The barb is an* individual rigid strand of feather material (keratin), extending laterally from the rachis to form the vane.

Barbule: The barbule is a lateral branch of a feather barb composed of two rows proximal and distal. The distally directed barbules have hooks that interlock with the proximal rows.

1.3.7 Microscopic Description of Feather

Fully developed feathers have a single cell layer of low cuboidal germinal cells with large spherical nuclei. Flattened and compacted cells are present above the germinal layer toward the feather lamina. Below the epidermal layer is a thin basement membrane (Lucas and Stettenheim 1972, p. 358). The dermis underneath the feather is dense and twice as thick as the epidermal layers. It is composed of collagen fibers with a few elastic fibers along with blood vessels and lymphatic vessels.

1.3.8 Types of Flying Feathers

Flying feathers (remiges) are divided in primary, secondary, and tertiary feathers. The tail feathers (rectrices) share general common structures in respect to the calamus and rachis. The primaries are usually 10 in number in chicken and curved inward, while the secondaries are 17–18 (Getty 1975, p. 2076) which are more curved inward compared to primaries. The numbers and shapes of tail feathers (rectrices) vary among breeds and between male and female.

1.3.8.1 Primary Remiges and Their Coverts (also Known as Cover Feathers)

The primary feathers are 10, stiff, pointed at the end and more symmetrical than rectrices. They start at the wrist as number 1 and end distally as number 10 at the edge of the wing. After feathers attach to the underside of the feather, usually at the level of the superior umbilicus. Coverts are present for both primary and secondary feathers (Figures 1.7 and 1.8).

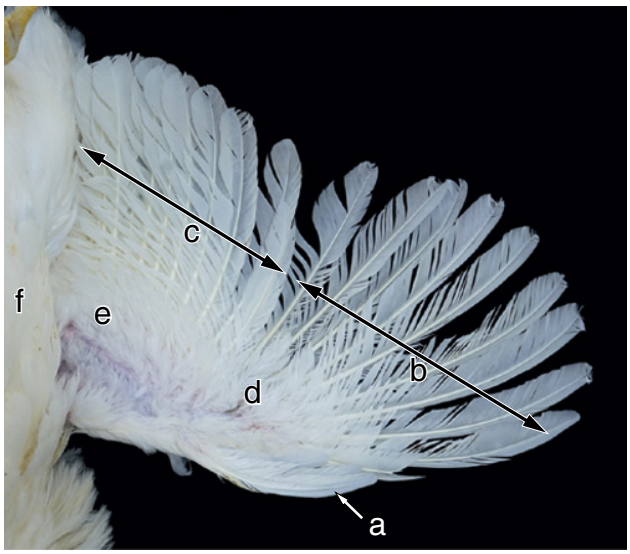


Figure 1.8 Wing feathers of a chicken. (a) Alulae, (b) primary feathers, (c) secondary feathers, (d) ventral covert for primary feathers, (e) ventral covert for secondary feathers, and (f) body contour feathers.

1.3.8.2 Secondary Remiges and Their Coverts

Secondary feathers in chicken are 17–18. The first 12 feathers are larger and stiffer compared with the last 6. Secondary covert feathers are arranged as upper major and upper median. They result in the formation of a distinct band (wing bar) (Getty 1975, pp. 2070 and 2077) (Figures 1.8 and 1.9A and B).

1.3.8.3 Rectrices and Their Coverts

Rectrices are sickle feathers surrounded by less curved tail feathers that vary between the rooster and the hen. It is stated that one through seven or eight rectrices implanted along the lateral margins of the tail. The bases of the tail feathers are attached to the pygostyle bone and covered by several rows of upper and under the tail coverts (major, median, and minor) (Getty 1975, p. 2076).

1.3.8.4 Contour Feathers

Contour feathers are the largest and the predominant type of feathers. The length and shape of contour feathers vary from region to region. Contour feather regions include the neck, thoracic, abdominal, pelvic limb, vent, and external ear opening. They have calamus and rachis plus inferior and superior umbilicus like flying feathers (Lucas and Stettenheim 1972, p. 253). Contour feathers consist of a well-developed shaft, a vane, and an afterfeather. The remige coverts (wing flight feathers), the rectrice coverts (tail flight feathers), and the general feathers of the body, neck, and limbs are all contour feathers. Replacement of contour feathers (molting) requires a longer period which may extend into three months (Nickel et al. 1977, p. 163) (Figure 1.10).

1.4 Lamellar Corpuscles

Large and small Herbst corpuscles were observed close to the feather and close to the skin surface (Figure 1.11). Each corpuscle is presented with an axis cylinder in the central part and is surrounded by outer and inner lamellae of concentric layers of connective tissue fibers. Reticular fibers were seen around blood vessels and in between adipose tissue (Bharathi et al. 2018). A small Herbst corpuscle serves as mechanoreceptor and registers slight movement which is present close to the filoplume, while a larger size corpuscle close to the calamus registers deep pain (Lucas and Stettenheim 1972, p. 276). Smooth muscles attach outside of the follicle by an elastic tendon in all feathers except in the filoplumes (Getty 1975, p. 2082).

1.5 Molting

Molting involves two phenomena, shedding of feathers (ecdysis) and growth of new feathers (endysis) (Lucas and Stettenheim 1972, p. 197; Watson 1963). The process starts after hatching by replacing the natal feathers with real feathers. Once all the feathers are fully developed, the first real molting starts. Both pituitary and thyroid glands play a significant role in the physiological molting

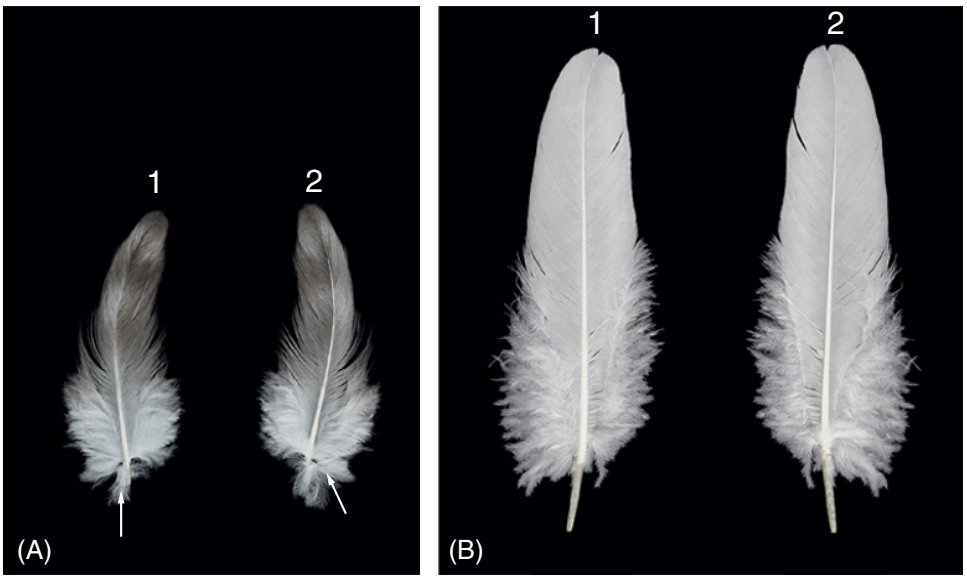


Figure 1.9 Relative size of feathers. (A) Primary feather covert from dorsal surface of the wing. Arrows indicate after feather. (B) Secondary feather covert from dorsal surface of the wing. (1) Dorsal surface of the feather and (2) ventral surface of the feather.

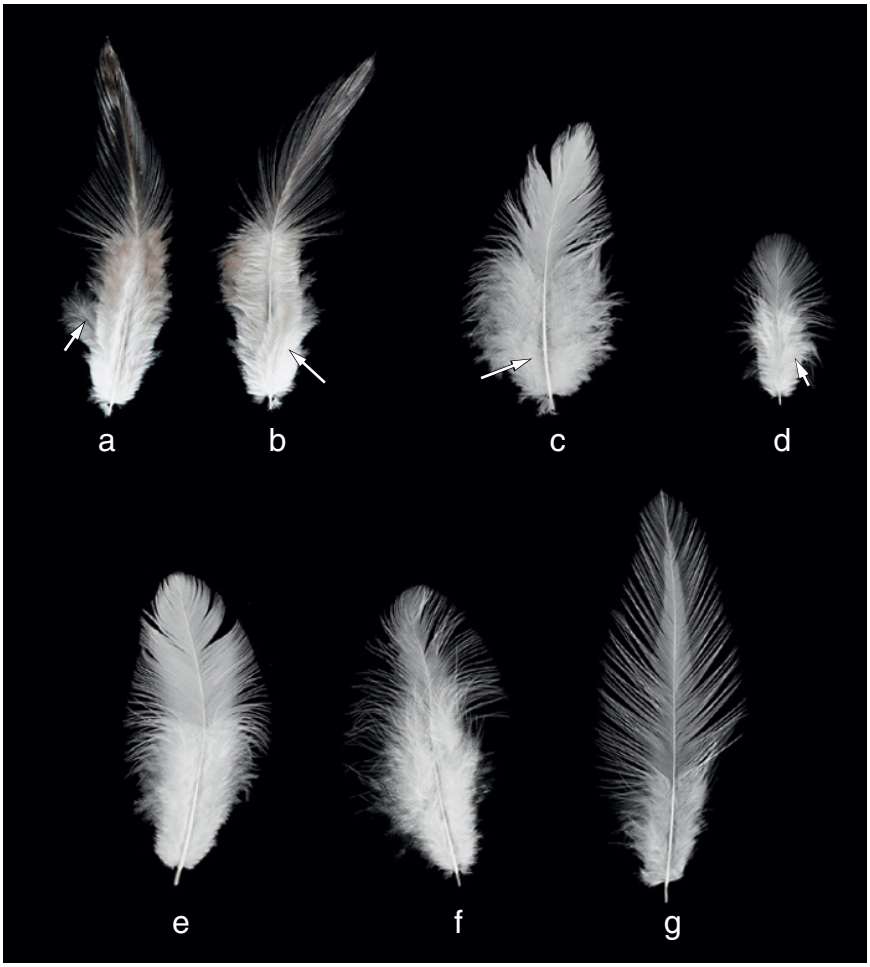


Figure 1.10 Relative size of contour feathers with after feathers (arrows) from different regions. (a and b) Colored feathers from the back, dorsal and ventral views, respectively, (c) dorsal chest region, (d) thigh region, (e) cranial abdomen, (f) caudal abdomen, and (g) neck.

process (Nickel et al. 1977, p. 162; Dyce et al. 2010, p. 789). Adult bird yearly molting usually happens at the end of summer or the beginning of the fall, lasting approximately for two months. For the primary feathers, molting starts from inside outward, while for the secondary feathers, it is the opposite. Filoplume molts along with its associated feather. Increased need for protein and other nutrients is essential for adequate feather molting. This is followed by a high metabolic rate and expenditure of energy. Diverse factors affect molting and timing like diet, environmental conditions, reproduction, season, temperature, humidity, lighting conditions, hormonal influences, species, and gender. Discrepancies of any of the listed factors often result in a partial molt or incomplete feather formation, which may even be manifested as a change in feather color (Perry 1987, pp. 40–50; Spearman and Hardy 1985, pp. 1–56). After completion of the feather development, there is always a quiescent papilla still at the base of the follicle. This papilla will always be ready to grow once the feather is shed or removed (Nickel et al. 1977, p. 163).

1.5.1 Induction of Molting by Food Deprivation

The commercial egg industry uses feed deprivation to induce molt because it is easy and gives the best results. Regardless of the results, feed deprivation and the stresses implied on the hens raise lots of concerns regarding animal welfare. At early stage of feed deprivation, hens have been seen to manifest temporarily increase levels of alertness, activity, and aggressive behavior during the first 48 hours. Alternative induced molting method has been sought to reduce animal welfare concerns. For example, the method that involves alteration of feeding regimen and cause at least some body weight loss (Webster 2003).

1.5.2 Feather Picking

Feather picking is common among chicken as well as other species of birds. Picking is usually around the tail, vent, or sometimes head. There are many reasons for feather picking in chicken, among them are overcrowded coop/housing, stresses of any kind, lack of proteins (reported to be rare by

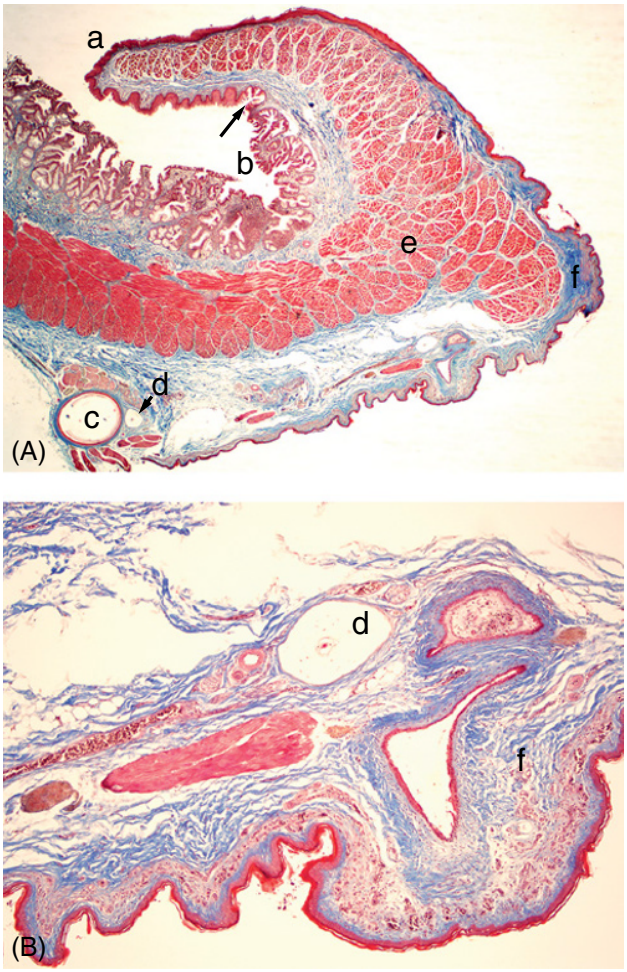


Figure 1.11 (A and B) Adult rooster vent showing the transition of the epithelium (arrow) from stratified squamous (a) to simple columnar epithelium inside the proctodeal cavity (b). Notice the presence of a feather follicle close to the skin (c) accompanied by Herbst corpuscle (d) and another Herbst corpuscle in the deeper dermis (d), vent skeletal muscle (e), and dermis (f).

Calder and Albright (2021)), excessive light, mineral deficiency, and parasitic infestation. Exposed areas of the skin encourage other chickens to pick. For the treatment of feather picking, one should consider the causative agent or its reason to eliminate first. This is followed by improving diet and paint window with red paints because chicken will go for the red exposed skin regions to pick. Individual experiences by the authors are the use of green alfaalfa hunged within the chicken housing to keep the chicken away from picking other chickens especially during the time of molting.

1.6 Debeaking

Debeaking (removal of one third of the beak) at one-day-old chicks is a widespread practice which showed no effect on body weight or mortality up to eight weeks of age (Lonsdale et al. 1957). Though positive results obtained from debeaking to stop cannibalism, stop feather picking, and decrease mortality, negative behaviors were reported (Cunningham 1992). The beak has nerve ending receptors and these are sensitive to pain (nociceptors). Trimming of the beak results in increased excitation of these nerve endings. Debeaking reported a decrease in activities for a brief period after the operation. Cunningham (1992) also reported that it is impossible to assess the degree of pain after debeaking, but the procedure can be considered painless. These comments were mentioned in response to the welfare activists who would like the breeders to stop debeaking because of the temporary pain the chicken exposed to during the operation and after it.

1.7 Eyelids Including Third Eyelid

Please see Chapter 9, Sense Organs (vision).

1.8 Vent

The vent is ellipsoidal in shape and consists of two labii, dorsal and ventral, which communicate with each other at the left and right commissures. The outer skin layers close to the labii are thin and consist of two to three layers of lightly keratinized stratified squamous epithelium. The stratified squamous epithelium becomes thinner at the junction with the simple columnar cloacal epithelium (Figure 1.12). The lamina propria and tunica submucosa are fused together due to the absence of lamina muscularis mucosa. This combined layer is called propria submucosa (Eurell and Frappier 2006, p. 172). The layer is filled with loose connective tissue, macrophages, lymphocytes, plasma cells, and fibroblasts. Coiled tubular glands line the most dorsal and middle part of the vent wall. They secrete mucus for lubrication and may play a role in certain species reproduction by helping to maintain favorable environment for the sperms. It is reported that the vent glands aid in extending the survivability of sperms (Dana et al. 2010). Two thick skeletal muscles surround the vent, inner circular and outer longitudinal. These muscle bundles are clearly surrounded by connective tissue of endomysium, perimysium, and epimysium. Individual myofiber surrounded by endomysium which are mostly reticular fibers, while the other two are mostly collagen fibers where perimysium surrounds a bundle of myofibers, while the epimysium surrounds the entire muscle. Bundles of small arteries, arterioles, and their accompanied veins and venules scattered within the connective tissue. Lamellated

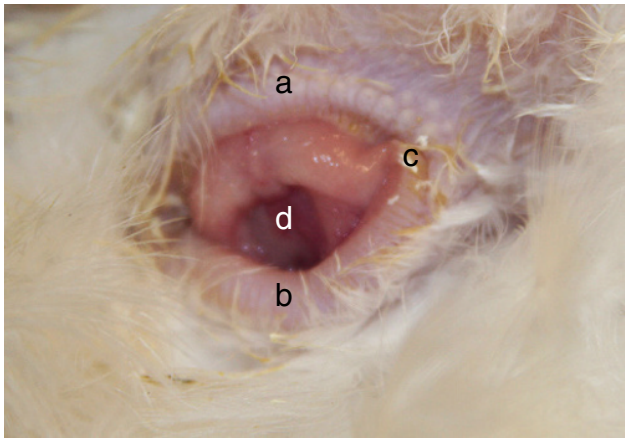


Figure 1.12 Vent of a hen. (a) Dorsal lip, (b) ventral lip, (c) commissure, and (d) cloacal cavity.

mechanoreceptor organs referred to as Herbst corpuscles in birds are present in several regions including the vent either deep or close to the papillae of the feathers (Figure 1.11).

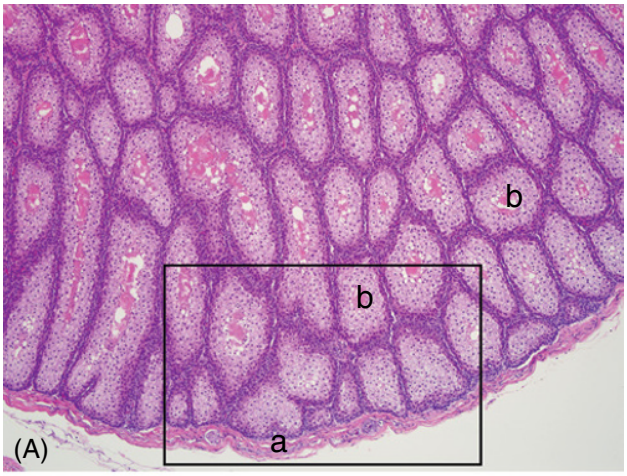
1.9 Uropygial Gland

The uropygial gland has several names including caudal, preen, or oil gland. It is present in chicken and very well developed in waterfowl. The gland is found at the dorsal base of the tail. Its secretion sustains feather condition and waterproofing. Its secretion also acts as a bacteriostatic, thus protecting the bird against bacterial infections Praveenkumar et al. (2023). The gland is a bilobed holocrine gland drained by a single papilla. The papilla is located dorsocaudally and is covered by a tuft of down feathers called the uropygial wick where the oily secretion is released outside the gland. This feather tuft may aid in transmitting oil from the gland to the beak while the chicken is preening. It is remarkably like the sebaceous glands of mammals. The uropygial gland is under androgen hormone secretion in songbird (Whittaker et al. 2018), Japanese quail (Abalain et al. 1984), and in domestic chicken (Hirao et al. 2009). The interaction between circulating testosterone and androgen receptor transcript abundance significantly correlates with the volatile oil concentrations in male, but not female preen oil. In both male and female, odorant variables correlate with aggressive response to an intruder which suggests that preen oil volatiles could function as signals of aggressive intent, and, at least in males may be regulated by local androgen receptor signaling in the uropygial gland (Whittaker et al. 2018). The secretion of this gland also has antibacterial and antimycotic properties as well as a potential odorant and/or pheromonal function (aids in the attraction of mates). The oil glands of some bird species like the hoopoes produce a foul-smelling liquid (specifically during breeding) that birds rub into their plumage. The unpleasant smell is believed to keep predators away from nesting females and their young as well as deterring parasites. The unpleasant odor of the secretions ceases just before the young fledge. The uropygial gland is reported to produce a sebaceous material having vitamin D precursors which are converted to the active form of vitamin D_3. During preening, the active form of vitamin D_3 is ingested (Birchard and Sherding 2006, p. 1765) (Figure 1.13).

Histologically, the uropygial gland is a branched alveolar holocrine gland. It is surrounded by dense irregular connective tissue capsule composed of collagen fibers and a few elastic fibers. The capsule sends trabeculae to divide each lobe into several alveoli (Figure 1.13). The capsule and the trabeculae are infiltrated by many blood vessels and nerve fibers. Each alveolus has a basement membrane adhering to it a single flattened cell layer forming the germinal epithelium. These cells are rapidly dividing to replace the shed cells toward the lumen of the gland. Germinal epithelium usually stained darker with routine stain (Hematoxylin and Eosin) compared to the cells above them. This difference in staining is the result of the cell layers above accumulating secretory products (lipids), thus changing color due to the removal of the lipid during processing of the tissue in organic solvents. The female's uropygial gland acts as a source of social odor cues in domestic chickens (Hirao et al. 2009).

1.10 Brood Pad

A brood pad is a patch on the midventral chest between the caudal sternum and pubic bones. During the breeding season, this patch loses feathers under the influence of estrogen and becomes thickened and vascularized to provide extra warmth during egg incubation. In some species like gulls, the number of brood patches is matched to the number of eggs in the clutch. However, lots of variations are mentioned in the literature about the relationship between the broods' patches and egg numbers in varied species of birds (Weibe and Botolloti 1993). Brood pad's blood supply arises from cutaneous branches of the external thoracic artery and from a cutaneous artery as a branch from the subclavian artery. These arteries are all accompanied by satellite veins (Nickel et al. 1977, p. 156).



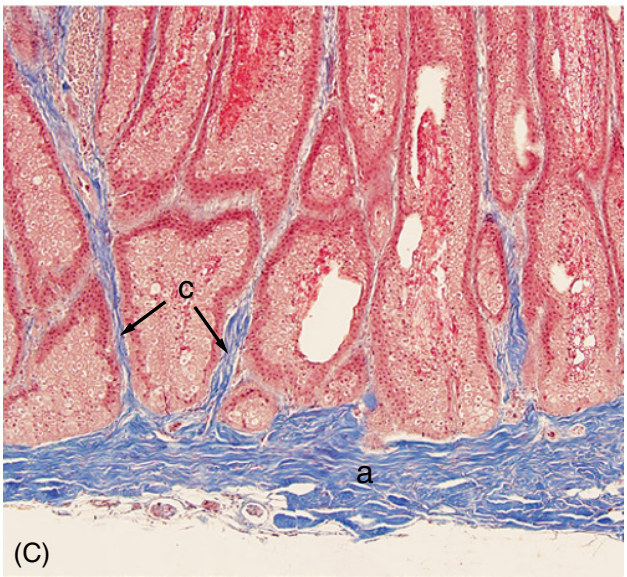


Figure 1.13 Histology photos of the uropygial gland of a chicken showing the capsule (a), acini (b) separated by thin layer of connective tissue, (c) arrows within the trichrome stained section, (d) lumen, and (e) basal cells (regenerative cells). (A and B) H&E stain. (C) Trichrome stain.

1.11 Bursa Sterni

The bursa sterni is at the cranial portion of the sternum. This bursa usually enlarges during brooding time to facilitate transfer of heat from the hen to the eggs and may sometimes get infected if injured.

1.12 Legs

1.12.1 Podotheca

The non-feathered area of the legs is called the podotheca and composed of keratinized epidermal plates the scales. The skin is thickened in the ventral metatarsophalangeal region and is designed to withstand impact on landing. In aquatic species, the skin is softer and more flexible and modified between the toes into webs. The distal phalanx is highly keratinized to form the nail or claw.

1.12.2 Scutes (Scales)

The scales are keratinized thickening of the outer layer of the epidermis. They are like the structural covering of the beak, spur, and claw. Thickness of the scales varies on the toes as they are thick on the dorsal surface and lateral, while they are thin on the plantar surface. The scales do not overlap in chicken. They vary in size, shape, amount of overlap, and degree of fusion on various parts of the foot. Parafibular nerve gives branches to the scaly skin of the lower leg. Mechanothermal receptors were detected in the scaly skin of the lower leg (Gentle et al. 2001) (Figure 1.14).

1.12.3 Digits

Several types of toe arrangement are present in different birds but the most common is an anisodactyl foot (chicken included); digit I points in a medial and plantar direction (the digit being turned backward), while the other three digits are spread out in a forward direction (Ryohei et al. 2012).

These digits are numbered from medial to lateral as I, II, III, IV, and V and they have, respectively, 2, 3, 4, and 5 phalanges (König et al. 2016, p. 68). Few breeds may have five digits (toes) like the Silkies. The terminal phalanges carry a claw. The chicken has two small webs joining the proximal portions of the digits. The one joining digits II and III is the intermediate interdigital web while the one joining digits III and IV is the lateral interdigital web. Small reticulate scales cover the surface of the webs (Lucas and Stettenheim 1972, pp. 67–69).

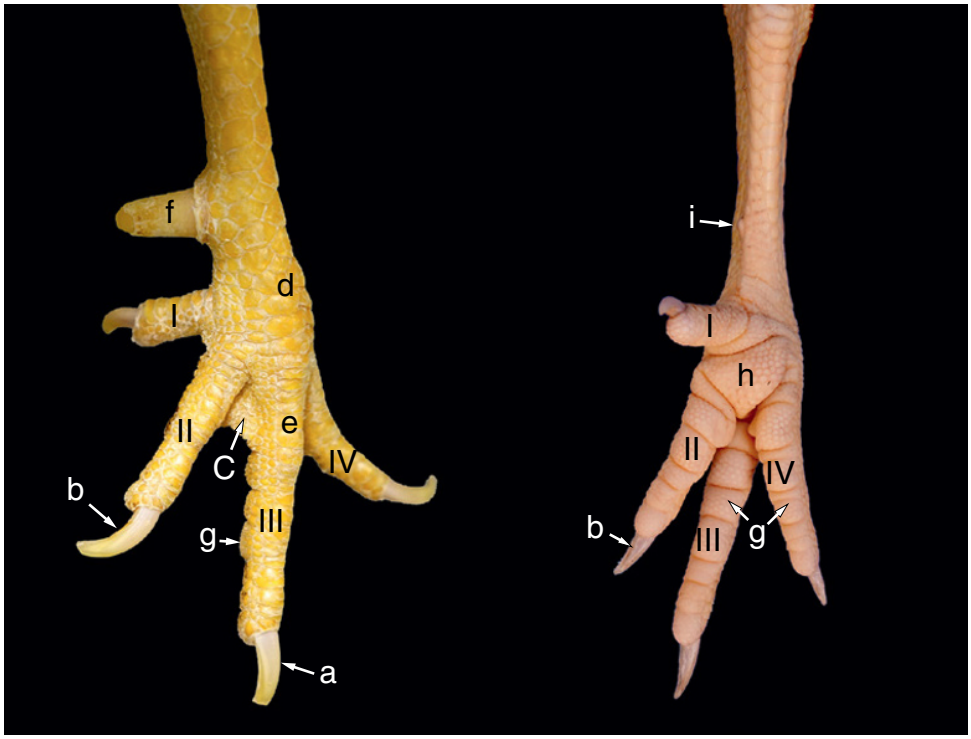


Figure 1.14 Dorsal view of a right metatarsal and digits of adult male chicken (left) and plantar view of a metatarsal and digits of adult female chicken (right). (a) Claw dorsal plate, (b) claw ventral plate, (c) medial interdigital web, (d) metatarsal scutes, (e) carpal scutes, (f) spur, (g) digital pad, (h) metatarsal pad, and (i) female spur. The Roman numbers indicate the number of the digits.

1.12.4 Claws

The claw is a type of hard keratin that extends from the end of the toe. It is short and serves the function of scratching or digging (Lucas and Stettenheim 1972, pp. 67–69) and fighting. The claw is laterally compressed. All claws in chicken have wide and thick base and they are short. They have dorsal and ventral plates. These claws vary in length between different digits. Claws are continuously replaced from inside out due to the continuous wear from outside (Hodges 1974, p. 14).

1.12.5 Spur

The spur is a pointed projection on the lower part of the tarsometatarsal region of the hind limb between the middle and distal parts of the tarsometatarsal bone. It is pointed caudomedially (Lucas and Stettenheim 1972, pp. 67 and 609). It is built on a small bony pointed prominence covered by a hard keratin. It is very well developed in male chicken and less developed in hens. Spur growth is measured yearly, and researchers may use it to estimate rooster age. It is covered by highly keratinized stratified squamous epithelium. Researchers found that overall spur size does not change after caponization (Doherty et al. 2021) (Figure 1.14).

1.12.6 Pads

1.12.6.1 Metatarsal Pad

A metatarsal pad is only one on each foot, bears weight, and is prone to injury with sharp surfaces or objects which results in what is called bumblefoot if swollen and/or infected. It is composed of substantial amounts of adipose and connective tissue, collagen, and a few elastic fibers covered by the epidermis with thick stratum corneum. It is highly vascularized with blood capillaries present close to the epidermis (Figure 1.15).

1.12.6.2 Digital Pads

The digital pads are soft tissue structures located on the ventral surface of all four digits. The number of pads conforms with the digit number in a way digit I has only one pad while digit IV has four pads. Digital pads conform with the underlying surface during perching (König et al. 2016, p. 255). They are covered by a thick keratinized stratified squamous epithelium with a short dermal papilla along with the epidermal pegs. The dermis is highly vascularized with medium size muscular arteries branching to fill the dense irregular connective tissue in the dermis with arterioles and capillaries distributed close to the epidermis.

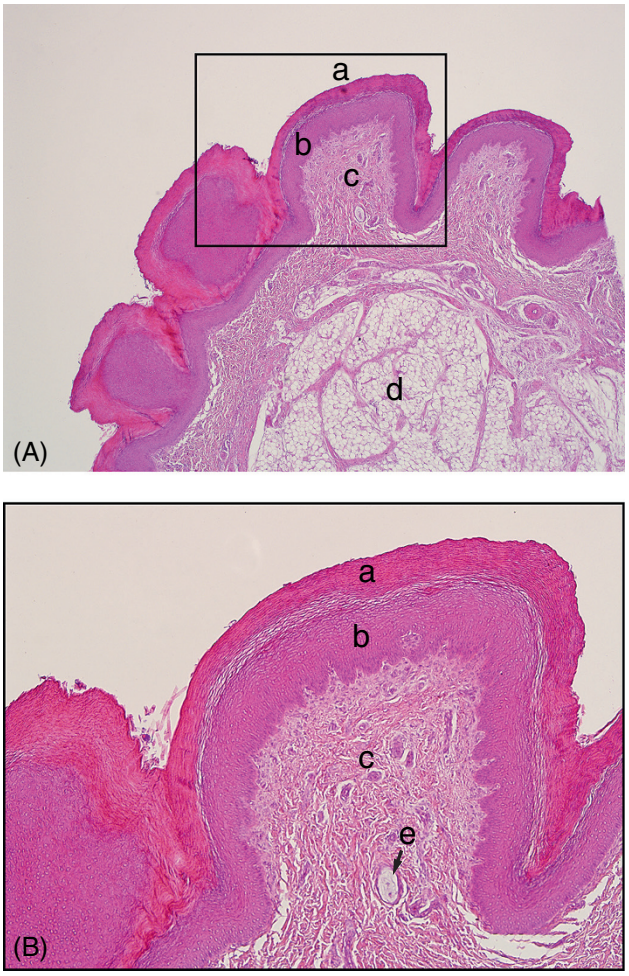


Figure 1.15 (A and B) Digital pad of an adult hen showing a highly keratinized stratified squamous epithelium, notice the thickness of the keratin (a), epithelium (b), dense irregular connective tissue mainly collagen bundles of the dermis (c), hypodermis (d), and Herbst corpuscle in the deeper dermis and blood vessels, hypodermis (e). H&E stain.

1.13 Thermoregulation

The thermoneutral zone of one-day-old chick is very narrow, like in all avian species. Birds, in general, are classified into three types according to certain abilities and external morphological characteristics. Precocial birds are those covered with down feathers when hatching such as the chicken, ducks, and geese. Thus, they are able to effectively respond to temperature changes. The second type is altricial and they hatch naked, need parental care, and have very limited ability to regulate their body temperature like pigeons, passerine birds, and raptors. The last type is an intermediate between the two.

During the last half of the incubation period, the fowl egg maintains a temperature above that of the ambient air temperature of 37–38 °C. When hatchlings are exposed to room air temperature, a new equilibrium is reached in about five hours (Tazawa and Rahn 1987; Tazawa and Whittow 2000). The difference between body temperature and ambient temperature increases from 6 °C immediately after hatching to 10 °C five days post-hatching. The newly hatched bird responds to temperature changes through increase in activity, such as movement of body parts or all of the body which requires conscious effort (Dawson and Whittow 2000). Shivering, as a thermoregulatory mechanism which uses neural thermoregulation, is available to birds at differing times following hatching (Dawson and Whittow 2000). Brooding is the process of providing supplemental heat in domestic poultry which simulates a similar response by the mother under natural conditions. Thyroid function is associated with thermoregulation because of its involvement with feather growth and basal metabolism and it has been used as basis to discriminate between precocial and altricial neonates (McNichols and Anne McNabb 1988). Thyroid hormone levels in precocial species peak prior to hatching and remain elevated during the first week after hatching, whereas in altricial species they peak following hatching. Weytjens et al. (1999) related plasma tri-iodothyronine concentrations to the onset of thermoregulation in high and low body weight lines of fowl. Greater tri-iodothyronine levels indicated earlier thermoregulatory abilities of the bird.

Wing spreading and panting are used to dissipate heat from the body under elevated temperature. When hens' combs and wattles are trimmed, there is an increment of panting and wing spreading due to the lack of ability to transfer heat from these structures to the environment. Trimming combs and wattles also affect egg weight. Pullets should not be subjected to a comb and wattle trim if they are housed in laying facilities that are not appropriately cooled during the summer. In addition, Al-Ramamneh et al. (2016) reported that trimming the comb and wattles of chicks at 21 days of age reduced adult feed usage. Also, as a thermoregulatory mechanism, it is reported that feathers trap air and function as insulator (Menon et al. 1996).

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2

Skeletal System

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2.1 Introduction

The skeletal system of avian species has modifications that differ from non-flighted species. These modifications include air diverticula in several bones to decrease their density, fewer joints, and fused bones. Chickens cannot fly, but these characteristic features persist within the skeletal system. One of most unique modifications to the skeletal system in the bird is that of the forelimb bones to form the wing. The hind limbs are also modified to carry the bird's entire weight on two limbs and allow for balance during walking, jumping, or flying. Certain bones, such as the humerus and synsacrum, have air diverticula that make the bones lighter. These pneumatized bones also have a trabecular network that allows them to keep their strength despite their lighter weight. The trabeculae get organized as the bird matures and stress is put on bones. Therefore, the arrangement of the trabeculae is unique to the stresses each bone receives throughout the lifetime of the bird. The major bone that protects the viscera of the bird is the sternum. It has well-developed processes with connective tissue connecting them. With a few chicken ribs, the connective tissue supports all the viscera from the ventral and lateral surfaces.

2.2 Cartilage and Bone Embryonic Origin

The mesoderm is the middle germ layer at the early stage of embryonic development. This layer is sandwiched between the outer layer of ectoderm and the inner layer of endoderm. The mesoderm splits into several regions including the somite, intermediate mesoderm, and lateral mesoderm. The cells that form the mesoderm are mesenchymal cells. These cells are pluripotent and can develop into fibroblast, chondroblast, osteoblast, myofiber, and blood cells. Therefore, cartilage and bones develop from the same layer. These embryonic (mesenchymal) cells disappear from the animal body with very few exceptions, like the pericyte around the blood capillary and arteriole.

2.3 Cartilage Cell Types

2.3.1 Chondrogenic (Chondroprogenitor)

Chondrogenic cells are present at the periphery of the hyaline cartilages, and they can develop into chondroblasts. They lie under the perichondrium (the connective tissue that surrounds the hyaline cartilage from outside).

2.3.2 Chondroblast

The chondroblast is the immature chondrocyte which develops from early mesenchymal cells (mesoderm). The cell keeps its high capability of dividing and developing into a mature chondrocyte.

2.3.3 Chondrocyte

Chondrocyte is a mature cartilage cell which secretes the chondroid material (territorial and interterritorial matrices). The matrix is composed of water, type II collagen, and proteoglycans which are prominent in hyaline cartilage, with lesser amounts of non-collagenous proteins and glycoproteins. The glycosaminoglycans form the proteoglycan unit (Cross and Mercer 2002, p. 84).

2.3.4 Chondroclast

The chondroclast is a multinucleated cell present in the region of calcified or degenerating zone of the hyaline cartilage. The cell is of monocytic lineage within the bone marrow and acts on the calcified cartilage region during bone

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development. Several histology textbooks do not mention chondroclast, and a few researchers described the cell as multinucleated which acts to resorb the calcified cartilage during the process of osteogenesis. They also indicated that monocyte lineage (macrophages) aids in the removal of the matrix from degenerating cartilage or damaged trabecular bone to facilitate the movement of the osteoblasts and osteoclasts to function in laying down of new osteoid materials (Włodarski et al. 2014, pp. 143–147).

2.4 Type of Cartilages

2.4.1 Hyaline Cartilage

Cartilage is avascular tissue which is nourished through diffusion from the close by blood vessels. It is surrounded by perichondral connective tissue (perichondrium) in hyaline and elastic cartilage. Growth in length and width of the cartilage is induced by the proliferation of chondroblasts and chondrocytes. Hyaline cartilage is present within the wall of the trachea, bronchi, upper larynx, articular surfaces of synovial joints, and epiphyseal cartilage when it is present in birds.

2.4.2 Fibrocartilage

Fibrocartilage can be identified when chondrocytes are present within dense connective tissue collagen bundles. Good examples of fibrocartilage in chicken are in the synsacrum and the menisci of the femorotibial joint. Fibrocartilage is present within the elastic long tendon of a specific region of the chicken wing. This is a distally located fibrocartilage from which fibrous connections extend to the capsule of the distal radius. In adult birds, this region shows the characteristics of tendon-compressed fibrocartilage, with an accumulation of proteoglycans between thick collagen bundles (Pimentel and Carvalho 2003).

2.4.3 Elastic Cartilage

No elastic cartilage is reported in chickens.

2.5 Cartilage Development

The cartilage within the animal body develops from mesenchyme. Mesodermal (mesenchymal) cells organize to form a cartilage model for future long bones. These mesenchymal cells proliferate to form chondroblasts that are surrounded by fibrous connective tissue (perichondrium). The cartilage model is always hyaline, which is avascular tissue. Once the cartilage is penetrated by a blood vessel, chondrocytes die and be replaced by osteoblasts. This process is called endochondral ossification. Alkaline phosphatase enzyme is identified as a marker for the differentiation and maturation of chondrocytes (Farquharson and Jefferies 2000). Enzymatic activity and morphometric measurements increase with incubation day, independent of the age of the hen. The process of endochondral ossification during the last two thirds of chick development is not influenced by the age of the animals (Alfonso-Torres et al. 2009).

2.6 Calcium Homeostasis

Long bone has an outer cortical layer and a medullary cavity with trabeculae. Prior to laying eggs, calcium is drawn from the alimentary tract and laid down by the endosteum inside the trabeculae. The total skeletal system increases in weight by about 20% prior to laying eggs. The process of adding bone tissue is called polyostotic hyperostosis and changes in bone density can be visualized radiographically. As the eggshell is calcified, bone resorption will be initiated (O'Malley 2005, p. 100) and osteoclastic activity will start.

Hens need lots of calcium to meet the eggshell's demand during its development. The calcium in the animal body is the main source to cover this process which may come from diet and medullary and cortical bones. Other mechanisms may also contribute to this effort like the decrease in secretion of calcium through the urinary system as well as the synthesis and degradation of the yolk which is continually happening during the laying period. Calcium mobilization and availability to the animal to form the eggshell fluctuate all the time because the process of shell formation needs calcium at different rates throughout the entire process (Etches 1987).

Calcium metabolism and requirements for bone growth are essentially like those of growing mammals. While hormones and regulating factors such as prostaglandins and calcitonin gene-related peptides affecting bone and calcium metabolism are different than mammals. This may be partly related to enhanced requirements of bone and calcium during eggshell formation (Whittow 2000, p. 473).

Laying eggs puts lots of strain on the calcium reserve within the hen body. Though the calcium demands are not continuous during the entire egg development. When the egg descends inside the magnum and isthmus, the medullary bone has many osteoblasts and osteoclasts. When the egg is in the uterus (eggshell gland), the medullary bone continues to have large numbers of both osteoblasts and osteoclasts during early calcification of the shell; as

calcification of the shell progresses, osteoblasts decrease and osteoclasts increase in number. In hens with good laying records, the medullary bone undergoes the sequences of bone formation and destruction connected with the storage and liberation of calcium (Etches 1987).

The demand for calcium may result in debilitating effect on the layer hens unless their diet is complemented with a calcium source. This is in addition to the maintained healthy physiological status of the hen like good quality water and least or no stresses of any kind. Bloom et al. (1958) demonstrated that changing the diet of egg layers to corn and sand, or corn, wheat, and sand resulted in stoppage of egg production with very few exceptions.

Parathyroid hormone maintains avian calcium levels. Its hypoglycemic effect is greater in egg laying hens than in cockerels due to either calcium binding by yolk protein with plasma or due to increased numbers of parathyroid hormone receptors which may be present in the medullary bone and the oviduct (Dacke 1979 cited after Whittow 2000, p. 475). Calcitonin secretion from the ultimobranchial body in chicken is regulated by rising plasma calcium levels leading to increased secretion of calcitonin from the C cells (Dacke 1979 cited after Whittow 2000, p. 475). Cholecalciferol (D_3) is a major class of vitamin D which is synthesized by animals. Chickens have a preference to use cholecalciferol (vitamin D_3) over ergocalciferol (vitamin D_2) (Chen and Bosmann 1965).

The bird has a unique gland (uropygial, preen) which is capable of secreting provitamin D3 that is converted into vitamin D3+ in the feathers. Removal of the preen gland from chicks causes rickets even when they are fed a normal diet and exposed to sunlight (Warren and Livingston 2021).

2.7 Bone Cell Types

2.7.1 Mesenchymal

Mesenchymal cells (mesodermal) are scattered all over the body and contribute to the formation of bone, cartilage, skeletal, cardiac, and smooth muscles in addition to the connective tissue and blood cells. Mesenchymal cells are pluripotent cells and can develop into all the cells mentioned depending on the environment of the specific site they are migrated into.

2.7.2 Pericyte

A pericyte is the cell present in fully developed animals outside the basal lamina of the blood capillary and arteriole. The cell has pluripotent capability and when any tissue damage occurs, the cell will detach off the wall of the damaged capillary and develop into one of the cells mentioned under mesenchyme depending on the growth factors and the environment of that site.

2.7.3 Osteogenic

Osteogenic cells are those with the capability to develop into an osteoblast. These cells are present below the outer layer of the periosteum of the bone. The periosteum has two layers, an outer fibrous layer and an inner cellular layer, also called osteogenic or osteoprogenitor layer. Both layers develop embryologically from mesenchyme.

2.7.4 Osteoblast

The osteoblast is an active immature bone cell and is highly mobile. The cell resides on the inner surface of the bone facing the medullary cavity. Therefore, it has easy access to the blood and nutrients. Osteoblasts mature into osteocytes.

2.7.5 Osteocyte

The osteocyte is a mature bone cell. It is surrounded by osteoid substance; therefore, it is immotile. The cell has many processes (extensions) extending within canaliculi of the hard osteoid tissue. Osteocytes contact each other and make connections with the surface osteoblasts. Through these connections, the osteoblasts can be activated to detach from their sites and form bone elsewhere due to the absence of sclerostin inhibition factor. Sclerostin is a glycoprotein produced by healthy osteocytes that suppresses bone formation (Delgado-Calle et al. 2017). It is an extracellular product that prevents the activation of osteoblast-mediated bone formation.

2.7.6 Osteoclast

The osteoclast is a multinucleated giant bone cell originating from blood cells through monocyte fusion. It functions in the removal of dead cartilage cells during initial stages of growth and in the continuous process of resorption and remodeling of the bone. It is present within the medullary cavity. It begins the process of bone resorption by anchoring itself on the defected region of bone creating a low pH pool, or lacuna (Howship's lacuna), where it secretes its lysosomal enzymes to remove the damaged bone by demineralization (dissolution of the inorganic part of the bone followed by enzymatic degradation of the organic matrix). Thus, it prepares the site for the osteoblasts to arrive and start building new bone (Liebich, 2019 p. 84).

2.8 Ossification

Centers of ossification in chicken and birds, in general, were studied by Hogg (1980) from hatching till the age of 182 days. The first ossification centers that appear around the time of hatching are for the uncinate processes of the ribs and phalanx IV. Ossification centers of the rest of the bones appear after hatching. Long bones in mammals have a primary center of ossification in the middle of the long bone (shaft) and two secondary centers, one at each extremity (epiphysis). However, these secondary centers are absent in the avian with one exception in which the proximal tibia has one secondary center. In this region, epiphyseal hyaline cartilage is present and surrounds the secondary center of ossification. The physeal cartilage includes the growth plate and the calcified cartilage between the cartilage and the metaphyseal region of the bone. Both the physeal and the articular cartilages are hyaline in nature. The physeal cartilage consists of resting, hypertrophic, proliferation, transitional, and resorption zones (Popescu 1997, pp. 22–23). Additional ossification centers are in the patellae and the carpal region (dorsal carpal sesamoid bone). The existence of two rows of ossification centers in the region of the avian hock joint is well known: the proximal fuses to the tibia and the distal to the great metatarsal.

2.8.1 Bone Formation (Ossification)

The trabecular bones in chicken are like those of mammals in respect to development and function. Their trabeculae are organized in a specific manner to provide the best support which results in maximum strength of the bone. The trabeculae distribution may change according to the stresses or the muscular pull during activities of the bird and reorganize to accommodate the needs for these changes. However, the compact part of the long bone is relatively thin compared to those bones in mammals (Figure 2.1). This thinness does not result in weakness of the bone because the trabecular arrangements mentioned before lend themselves to support weight and manage the stresses resulting from bipedal ambulation in the bird.

There are two types of ossifications in birds like those in mammals, intramembranous and endochondral. Intramembranous is when the bone cells develop within the mesenchyme without the presence of a cartilage model. Endochondral ossification requires a cartilage model first

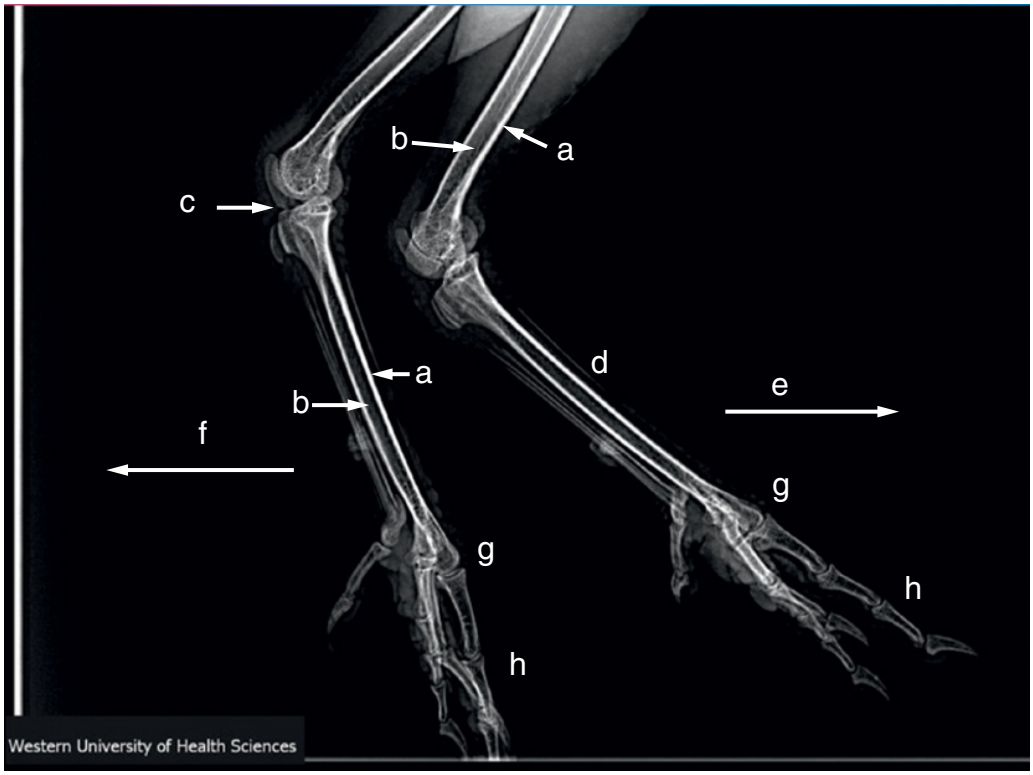

Figure 2.1 Hindlimbs radiograph of a chicken showing the (a) radiopaque outer layer and (b) larger radiolucent medullary part of the compact bones (c) tibiotarsus articulating with (d) tarsometatarsus through the intertarsus joint, (e) cranial, (f) caudal, (g) metatarsophalangeal joint, and (h) phalanges.

followed by introduction of a blood vessel; therefore, the chondrocytes die and are replaced by osteoblasts.

2.8.2 Intramembranous Ossification

2.8.2.1 Flat Bones

Flat bones start to develop early in birds between days 9 and 14 for the membranous flat bones in the skull. Mesenchymal cells migrate to the head region to populate the head and the sites where future flat bones will develop. These mesenchymal cells form a meshwork like any connective tissue elsewhere in the animal body. These cells proliferate into osteoblasts which, in turn, mature into osteocytes within the future flat bone. The periphery forms the perichondrium which is connective tissue covering the outside of the bone. The perichondrium is composed of fibroblasts/fibrocytes and functions as a route for passing blood vessels, lymphatic, and nerves.

2.8.3 Endochondral Ossification

2.8.3.1 Long Bone

General anatomical directional terms used are remarkably like those of mammals, with several examples labeled on the articulated chicken skeleton (Figure 2.2). Different anatomical regions of the long bone are described in Figure 2.3. Long bone has proximal and distal epiphyses, metaphyses, and diaphysis or shaft of the bone. Although bones stop growing after they reach maximum length, they will continue to grow throughout the bird's life in response to stress and muscular activities.

In birds, at hatching, the initial cartilage plate of the long bone is penetrated by vascular canals from the epiphyseal and diaphyseal regions. Growth of the cartilage through proliferation of the chondroblasts results in thinning and reduction of the blood vessels. Epiphyseal and metaphyseal blood vessels continue to supply the growth plate. In the duckling proximal femur, the vascular canals cross

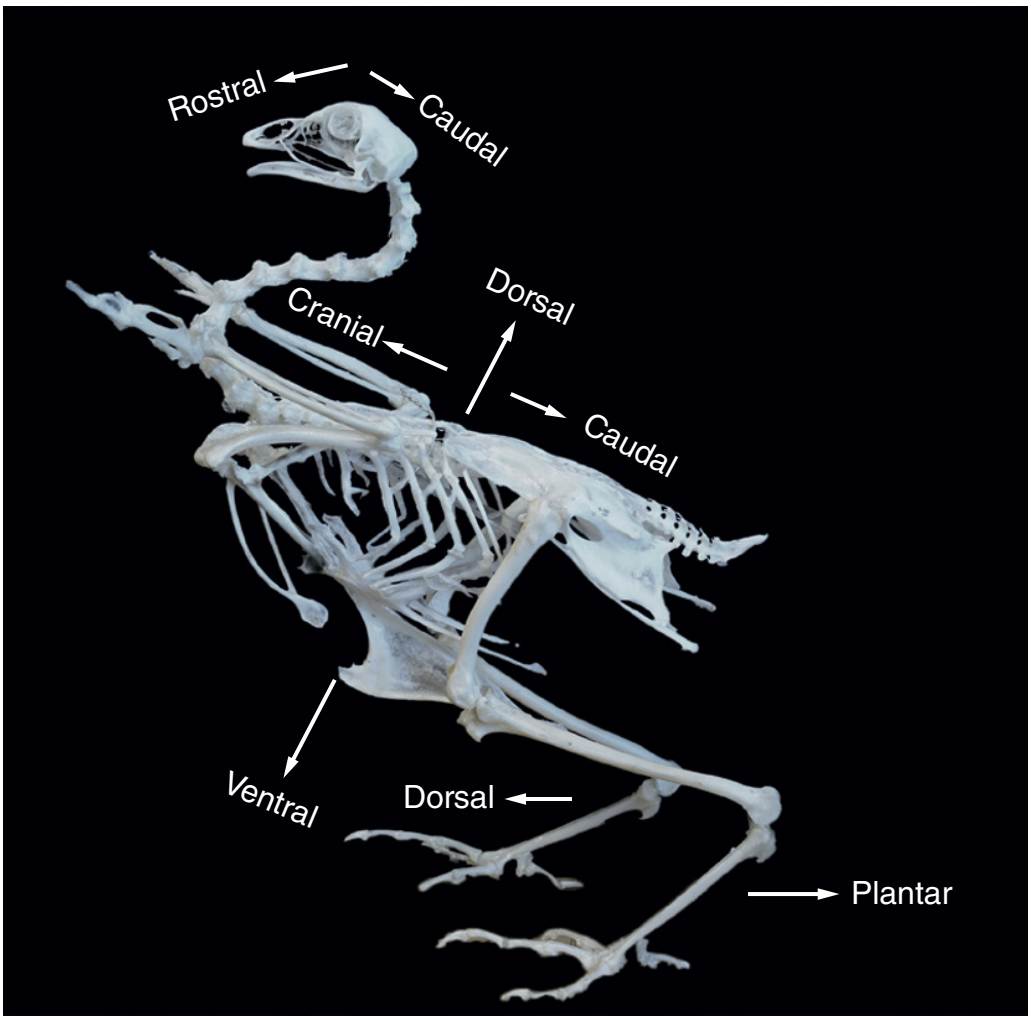

Figure 2.2 Chicken skeleton showing the directional terms.

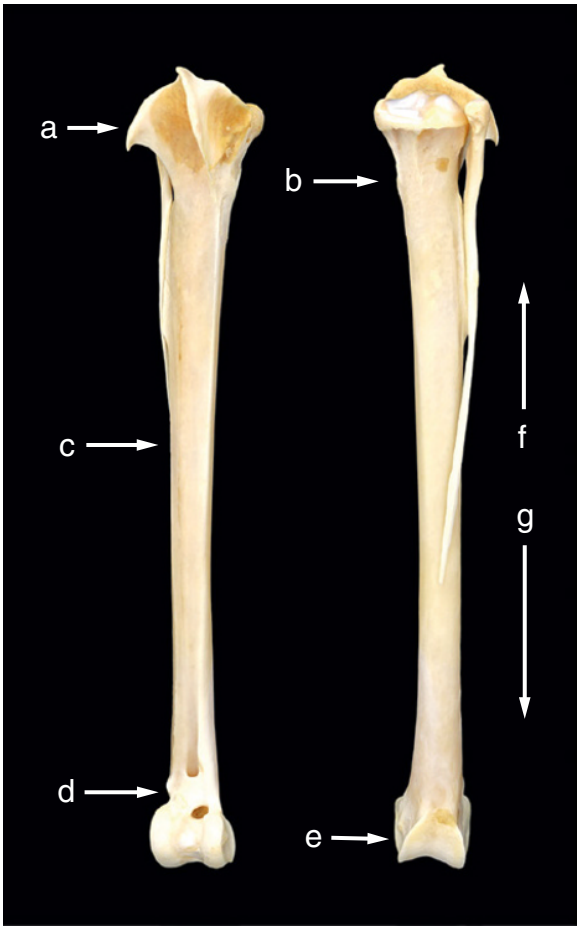

Figure 2.3 Different anatomical regions of a long bone (example used is tibiotarsus) show (a) proximal epiphysis, (b) metaphysis, (c) diaphysis, (d) distal metaphysis, (e) distal epiphysis, (f) proximal, and (g) distal.

the growth plate from the lateral surface. The canals disappeared after the 8th week of life through isolation of the growth plate (Popescu 1997, p. 42).

Long bone starts to develop at the early stages of chick development. All bones originate embryologically from the mesoderm (mesenchymal, pluripotent cells). Genetic and local factors induce the mesenchymal cells into a cartilage model for the future long bone. Inducing factors result in having the mesenchymal cells develop into chondroblasts, while the periphery of the bone model is connective tissue forming the perichondrium. These chondroblasts continue to proliferate and lay down the cartilage matrix (chondroid substances among other substances over a collagen meshwork) and mature to be chondrocytes. This process is limited by several factors to stop proliferation when reaching the final shape of the developing bone. At the middle of the cartilage, blood vessels penetrate the cartilage model. Hyaline cartilage is avascular and gets its nourishment through diffusion. As the minute blood vessels enter the cartilage, the chondrocytes and chondroblasts start to degenerate. Dead tissues and debris from the cartilage model are removed by multinucleated chondroclasts. The penetrating blood vessels bring a pluripotent cell, the pericyte, which differentiates into an osteoblast. The osteoblast, in turn, starts to lay down the osteoid material to harden the tissue and create new bone. The osteoblast continues to proliferate and produce bony matrix starting from the periphery of the cartilage model (perichondral ossification) to reach the middle part of the model (König et al. 2016, p. 17). This early locus of ossification is called the primary center of ossification. The ossification starts to move proximally and distally to ossify the shaft of the long bone while the chondrocyte/chondroblast dies and is removed by phagocytosis. There are no clear secondary centers of ossifications in birds other than in the tibiotarsal region.

In one study, growth plates appeared at the base of the intramedullary cartilage cone at each end of the long bones at the 16th day post-hatching and persisted until the end of growth at 30 weeks after hatching. True bony epiphyses (i.e., secondary centers of ossification) developed in the proximal end of the tarsometatarsal bone and the proximal and distal ends of the tibiotarsal bones. They appeared soon after hatching and fused with the diaphysis between 14 and 18 weeks of age. Bony epiphyses developed in bones that showed the most rapid rates of growth in length (Church and Johnson 1964). The average weight, length, size (thickness) of marrow cavity, and cortical bone thickness in all the bones, gradually increased with the age of the broiler birds under study. The diameter (width) of all the bones in broiler birds showed a steep increase from day 7 up to day 21 and a steady increase thereafter until day 42. Though initially the mid shaft cortical bone was thinner than the epiphyseal ends, it gradually became thicker from day 21 (in femur and tarsometatarsus) or from day 28 (in tibiotarsus) until day 42. The gross morphological observation did not reveal the presence of growth plate cartilages in all day-old chicks. Morphometrical parameters (dimensions) of the long leg bones like length and width were good and reliable indicators of musculoskeletal growth in broiler chickens.

At the early stage of ossification, the medulla of a long bone is filled with red marrow. This tissue changes to yellow marrow with age (König et al. 2016, p. 19). Male skeletons are heavier, and female skeletons mature earlier. The growth in weight continues after the growth in length ceases (Latimer 1927).

2.9 Axial Skeleton

2.9.1 Skull

Most of the skull bones have dual embryonic origin cartilaginous (endochondral) and membranous (intramembranous). Flat bones grow by marginal ossifications at the future suture sites between adjacent bones. At the early stage of development, skull bones fuse together to form one structure (Chamberlain 1943, p. 11) (Figure 2.4). The skull bones vary from the start of ossification until the elements take their final shape in their developmental maturation. The elements of the lower jaw and ventral side of the skull begin ossifying before the skull roof and most elements take roughly five days to reach their final shape, while others take up to nine days (frontal) (Arnaout et al. 2021). The chicken has a single occipital condyle that articulates with a small ring-shaped atlas. Chickens have large eyes, thus large orbital cavities present within the skull to accommodate that eye size. The two bony orbits are separated by a thin bony plate called the interorbital septum. The quadrate is a very complex bone that articulates with the mandible to help in jaw suspension. It also forms the pivotal bone for the kinetic jaw mechanism (Khamas and Rutllant-Labeaga 2021, pp. 134–136).

2.9.2 Vertebrae

The chicken has 16 cervical vertebrae. The articulations between the vertebrae are synovial and the articular surfaces of the bodies are saddle-shaped with fibrocartilage.

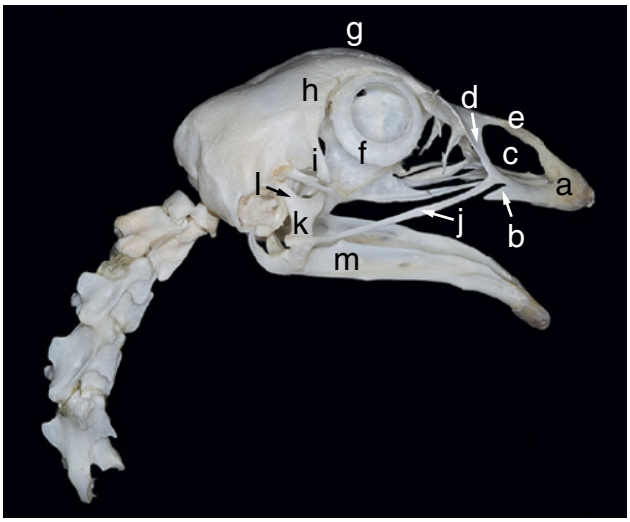

Figure 2.4 Chicken skull lateral view (a) premaxilla, (b) maxilla, (c) opening of external nares, (d) lateral ramus of the nasal bone, (e) nasal bone, (f) scleral ring, (g) frontal bone, (h) parietal bone, (i) postorbital process, (j) jugal bar, (k) quadratojugal bone, (l) squamosal bone, and (m) mandible.

These anatomical features give great flexibility to the vertebrae (McLelland 1991, p. 36). There are seven thoracic vertebrae with the first and second vertebrae having asternal ribs. These ribs are directed caudoventrally. The third to the seventh have sternal ribs. The second to fifth are fused forming the notarium. Thoracic vertebrae are partially fused with the caudal cervicals to form the notarium or with the lumbar and sacrals to form the synsacrum. The chicken has five to six well-developed caudal vertebrae, not in retrogression like in those of mammals. The last two vertebrae are fused to form the pygostyle which contributes to the movement of the tail follicles (Koch 1973, pp. 24, 27).

2.9.3 Notarium

Five fused thoracic vertebrae form the notarium in chicken (Figure 2.5). The number of fused vertebrae may vary in other avian species. It may fuse directly with the following vertebral segment of one to three free vertebrae followed by the next complex (Koch 1973, p. 26). However, McLelland (1991, p. 36) stated that in adult chicken, the notarium is formed by fusion of the last cervical and the

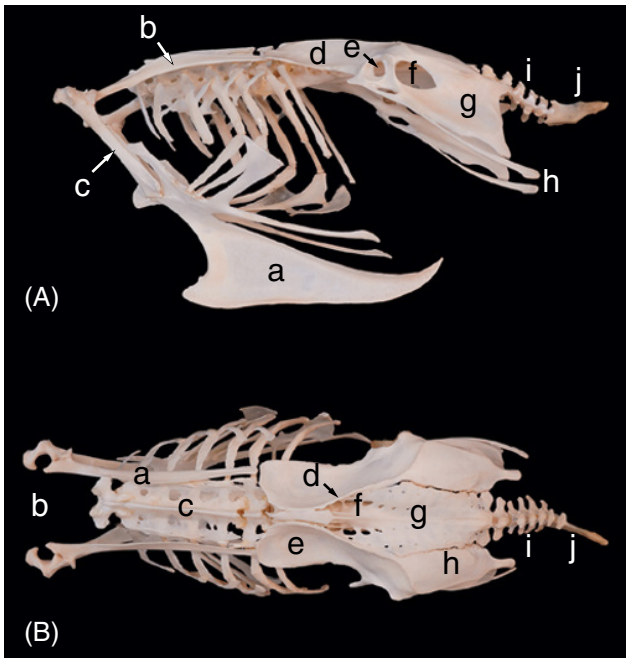

Figure 2.5 Shoulder girdle and the sternum articulating with the synsacrum and caudal vertebrae (A) left lateral view, (a) sternum, (b) scapula, (c) coracoid, (d) ilium, (e) acetabular foramen for femur articulation, (f) ilioischiatic foramen, (g) ischium, (h) pubis, (i) caudal vertebrae, and (j) pygostyle and (B) dorsal view, (a) scapula, (b) triosseal foramen for the passage of the supracoracoideus muscle, (c) notorium, (d) dorsal iliac crest, (e) preacetabular ilial wing, (f) fenestrae intertransversaria, (g) synsacrum, (h) postacetabular ilial wing, (i) caudal vertebrae, and (j) pygostyle.

first three thoracic vertebrae. The fusion starts at about four months of age.

2.9.4 Ribs (Costae)

In chicken, there are five to six pairs of ribs (McLelland 1991, p. 37). The bird has asternal and sternal ribs. Asternal ribs are present in the cranial and caudal thoracic regions (Figure 2.6). They consist of single bones articulating proximally with the body and transverse processes of the vertebrae while the most caudal ribs are free within the back musculature. The sternal ribs consist of two pieces of bone articulating with one another. The vertebral part of the rib articulates with the vertebra and carries the uncinate process while the sternal part (corresponds to the rib cartilage in mammals) lies at an angle to it. The capitulum and the costal tubercle are fully developed and separated from each other (Koch 1973, p. 28). The uncinate processes of the sternal ribs supply attachment for muscles and ligaments and strengthen the thoracic wall.

2.9.5 Sternum

The sternum is very well developed in birds and consists of the main single flat bone showing no segmentation (no sternebrae) in chicken. The lateral broad surface forms the carina or keel bone. The keel bone is the main attachment for the flight muscles (supracoracoideus and pectoralis) (Figure 2.7). The poultry sternum has notched or perforated windows due to lack of complete ossification. These perforations are lined by a membranous connective tissue (Koch 1973, pp. 37–38). The sternum has a body with a dorsal concave plate and a ventral sharp projection, the keel, forming its ventral edge for the attachment of the flight muscles. The carina has a sharp cranial projection that forms the apex, and the most caudal part forms the median trabecula. Dorsally, the sternum has a thicker median projection which is the sternum rostrum. Two (left and right) craniolateral projection processes form the cranial border of the ribcage. There are two projections on each costo-lateral surface of the sternum for the articulation with the distal ends of the sternal ribs (costal margin). The dorso-lateral projection is the caudolateral process (lateral trabecula) with the most caudal projection. The caudo-dorsal projections are the caudomedial process (intermediate trabecula). These two pairs of projections border the lateral incisure. The medial incisure is formed by the medial and intermediate trabeculae. Both incisures are closed by connective tissue in the live bird (Nickel et al. 1977, pp. 10–11). The visceral surface of the sternum has a cranial cardiac part and a larger hepatic part. The clavicular air sac pneumatizes the sternum through laterally located pneumatic pores and one cranial pneumatic foramen (König et al. 2016, p. 37).

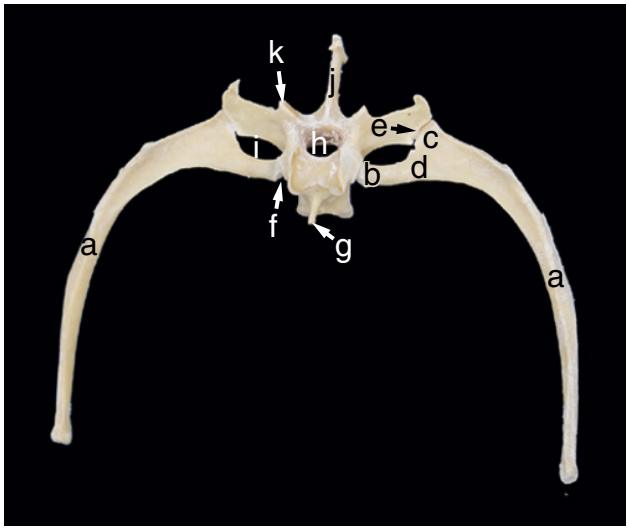

Figure 2.6 Thoracic vertebra with a pair of ribs attached to it showing (a) vertebral part of the rib, (b) rib head (capitulum), (c) costal tubercle, (d) neck of the rib, (e) articular surface for the tubercle, (f) articular surface for the rib head, (g) ventral vertebral crest, (h) vertebral foramen, (i) vertebral notch, (j) spinal process, and (k) articular process.

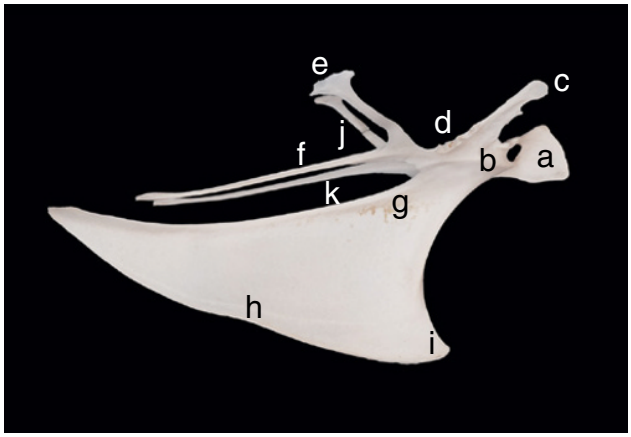

Figure 2.7 Chicken sternum showing (a) manubrial spine (sternum rostrum), (b) foramen (notch) of the manubrium, (c) sternocoracoidal (cranial lateral) process, (d) costal surface (serrated), (e) caudolateral process, (f) caudomedial process, (g) lateral surface of the keel, (h) carina (sternal keel), (i) apex of carina, (j) lateral incisure, and (k) medial incisure.

2.10 Sesamoid Bones

The patella is a well developed sesamoid bone. It is a thick structure and often present in the knee. An additional sesamoid bone is the bony carpal sesamoid bone present close to the radial carpal bone (Hogg 1980). No visceral (splanchnic) bones are reported in chicken.

2.11 Pneumatic Bones

It is reported that the cervical vertebrae are aerated except the atlas and the axis, the thoracic vertebrae except the fifth, the lumbosacral mass, the pelvic girdle, the first two vertebral ribs, the plate and the cranial processes of the sternum, the humerus, and the distal half of the coracoid. The head is pneumatized but not by the air sacs extensions (King 1957). While Hogg (1984) reported that pneumatization is regularly present throughout the neurocranium and in the quadrate bone, and variably in the mandible, it is absent in the facial skeleton. Pneumatization of cervical vertebrae 5–9 is regularly reported. The humerus and coracoid are variably pneumatized, often unilaterally (Figure 2.8). The os coxae and sternum have extremely low incidence of pneumatization. Cockerels appear to be relatively well pneumatized.

The air sacs, in general, consist of extremely thin membranes which are composed of a simple cuboidal epithelial cells resting on a basal lamina with a small amount of connective tissue (for more information see Chapter 5, Respiratory system). Air sacs are poorly supplied with blood; thus, no air exchange takes place within the air sacs nor within their extensions. Air sac extensions pneumatize different bones. In general, bones like the humerus, coracoid, pelvis, sternum, and cervical vertebrae are usually pneumatized in birds. Other bones may be pneumatized like the femur (in ostrich), the scapula, and the furcula (Koch 1973, p. 46).

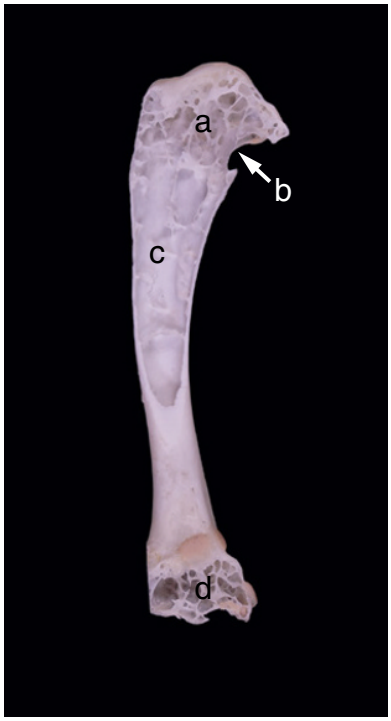

Figure 2.8 Left chicken humerus, (a) caput (head) of the humerus with (b) pneumatic foramen, (c) humeral diverticulum within the shaft of the humerus, and (d) distal extremity showing air-filled spaces.

Many modifications of the avian skeleton and other systems is to allow for decreased weight to aid in flight. It is thought that pneumatization is one mechanism for decreasing total body weight, though it is well established that pneumatization plays a vital role in transmission of infections/diseases from the respiratory system to the skeleton in domestic poultry.

Greater the circumference of these bones due to greater volume and a relatively smaller proportion of bony tissue compared to non-pneumatized bone give them a greater resistance to bending strains, despite the thinness of the wall. Small birds are poorly pneumatized compared to richly pneumatized large birds irrespective of their powers of flight (Marshal 1960, pp. 289–293).

The post-cranial skeleton becomes pneumatized by extensions from the air sacs system; cervical vertebrae from the cervical air sac, the wing, and shoulder girdle from the clavicular sacs, and the pelvis, synsacrum, and femur from the caudal (Marshal 1960, pp. 289–290).

2.12 Pectoral Girdle

2.12.1 Scapula

The scapula is found dorsolateral to the thorax paralleling the vertebral spine. It is a flattened long bone and curved mediodorsally. It has two surfaces, two borders, and two extremities (Figure 2.9). The cranial extremity has a tuberosity on its medial surface which forms the medial boundary of the triosseal canal. The coracoid articulates on a rounded convex articular surface lateral to the tuberosity. A similar concave articular surface together with the articular surface of the coracoid bone forms the glenoid cavity. This cavity articulates with the humerus (Chamberlain 1943, p. 2). One of the key factors for flight in birds is the presence of the glenoid cavity of the scapula which makes it possible for adduction and abduction of the forelimb (King and McLelland 1984, p. 29).

2.12.2 Coracoid

The paired coracoid bone is rod-shaped and the largest bone of the pectoral girdle on the craniolateral aspect of the thorax (Figure 2.9). The triosseal canal is found at the superior end of the coracoid bone (Proctor and Lynch 1993, p. 134).

2.12.3 Clavicle

The clavicle is a long and thin bone that makes the V-shaped furcula (commonly known as wish bone) of the breast.

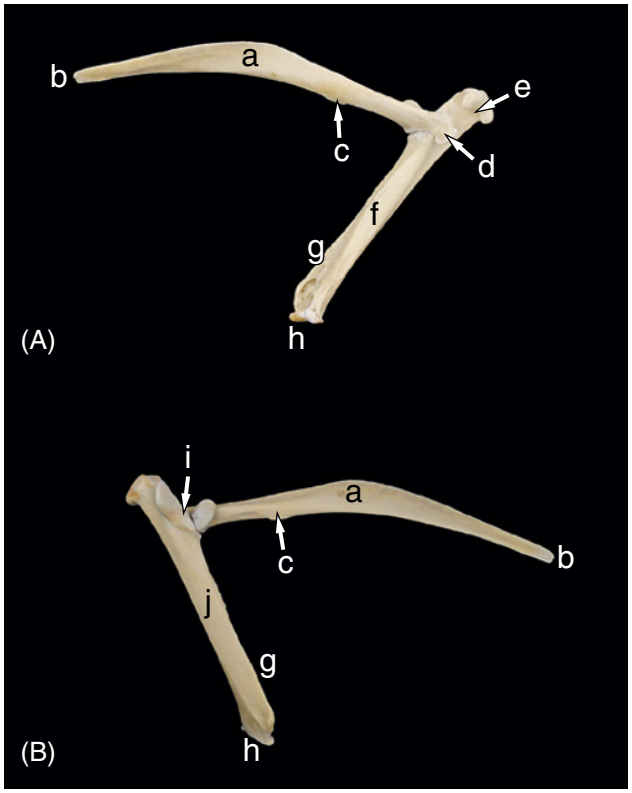

Figure 2.9 Pectoral (shoulder) girdle showing the scapula and the coracoid bones, (A) medial view and (B) lateral view, (a) scapula, (b) caudal extremity of the scapula, (c) scapular tubercle, (d) scapular tuberosity, (e) articular surface for the clavicle, (f) medial surface of coracoid bone, (g) caudal surface of coracoid bone, (h) distal extremity of the coracoid, (i) glenoid articular surface, and (j) lateral surface of coracoid bone.

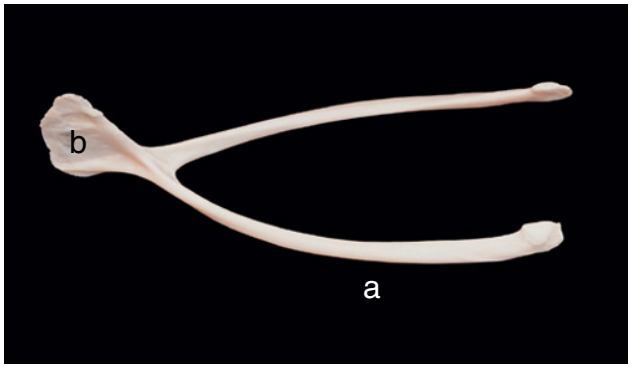

Figure 2.10 The chicken furcula shows (a) ramus and (b) hypocledium.

The hypocledium (body) is flattened laterally and circular saggitally (Figure 2.10). It attaches to the sternum by the acromioclavicular ligament (fibrous joint). The distal extremity of the clavicle articulates with the distal extremity of the coracoid bone (fibrous joint). Its medial plate joins the sternum rostrum, either directly or by fibrous connection. The dorsal forked points join the scapula and the coracoid by fibrous connective tissue (Koch 1973, p. 31). The clavicle is partially pneumatized in some birds, although not in chickens.

2.12.4 Triosseal Foramen

The triosseal foramen (canal) is formed by the scapula, clavicle, and the coracoid bones (Figures 2.5B and 2.9). The tendon of insertion of the supracoracoideus muscle passes through the triosseal foramen to elevate the humerus during upstroke phase of flight (Rayner 1988). The supracoracoideus muscle produces elevation and supination of the wing by a long tendon that passes dorsally over the shoulder, via the triosseal foramen before attaching to the dorsal surface of the proximal humerus (Biewener 2011).

2.13 Pectoral Limb (Wing)

2.13.1 Humerus

The humerus has a head (caput), shaft (diaphysis), and two extremities (proximal and distal) (Figure 2.11A and B). It articulates proximally with the glenoid cavity of the scapula by a large convex head. At the head, the humerus has a

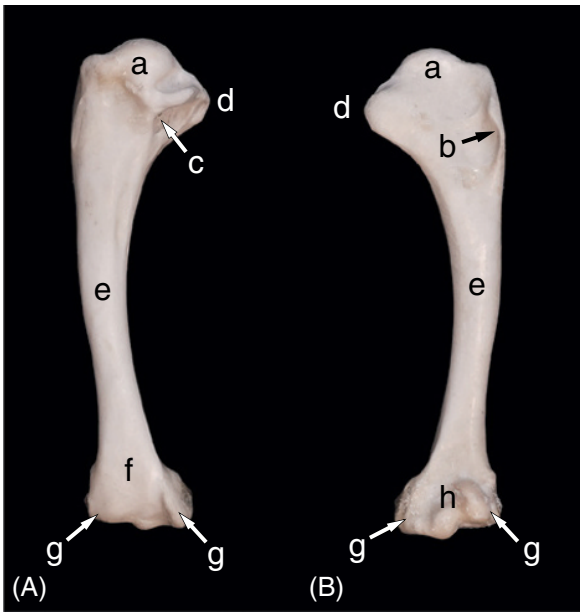

Figure 2.11 (A and B). Left medial and lateral chicken humerus, (A) medial view and (B) lateral view. (a) Caput (head) of the humerus, (b) deltoid tuberosity, (c) pneumatic foramen, (d) medial proximal tuberosity, (e) humeral shaft, (f) olecranon fossa, (g) humeral condyle, and (h) intercondylar groove.

pneumatic foramen which communicates with the clavicular air sac. The chicken humerus has a deltoid crest where the pectoralis muscle inserts ventral to it. The distal extremity of the humerus has medial and lateral condyles with an intercondyloid groove and an olecranon fossa (Getty 1975, pp. 1796–1797). Two epicondyles (lateral and medial) are present on the distal extremity of the humerus for muscular attachments.

2.13.2 Radius and Ulna

The ulna is larger than the radius in the chicken. This is the opposite of mammals. The two bones are separated by a large interosseus space. Both bones are of equal length (Figures 2.12 and 2.13). They articulate proximally with the humeral condyles and distally with the carpal bones. The distal radius articulates with the ulna and the carpus, while the ulna articulates with the radial carpal and ulnar carpal bones (Getty 1975, p. 1798). The proximal part of the ulna has the olecranon process which projects above the articular surface of the larger condyle of the humerus.

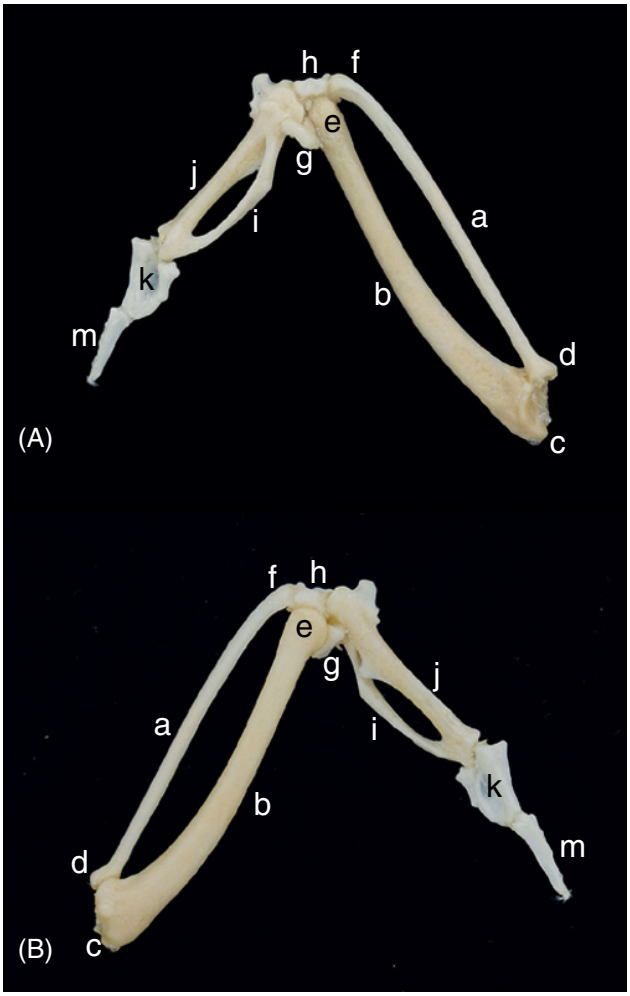

Figure 2.12 Right chicken wing without the humerus, (A) medial view and (B) lateral view. (a) Shaft of radius, (b) shaft of ulna, (c) ulnar olecranon, (d) proximal extremity of the radius, (e) distal extremity of the ulna, (f) distal extremity of the radius, (g) ulnar carpal bone, (h) radial carpal bone, (i) minor metacarpal bone, (j) major metacarpal bone, (k) proximal phalanx of major digit, (l) phalanx of minor digit, and (m) distal phalanx of major digit.

2.13.3 Carpometacarpal

The carpometacarpal comprises only three elements, metacarpal II, III, and IV because metacarpal I is absent in the chicken. The third digit is the largest with two phalanges, while II and IV have one phalanx (Figure 2.12). That is in addition to the rudimentary distal phalanx for all digits (Getty 1975, p. 1798) which is pointed like a spine in all phalanges (Koch 1973, p. 33). Metacarpal II is the strongest bone compared to other metacarpals.

2.14 Pelvic Girdle

The pelvic gridle consists of three bones like the mammals (ilium, ischium, and pubis). The ilium fuses with synsacrum. The pubis fuses with the ischium and the obturator foramen separates the two bones cranially. An additional foramen, the ilioischiatic foramen, is present between the ilium and the ischium (Getty 1975, p. 1798).

2.15 Pelvic Limb

2.15.1 Femur

The femur has a head (caput), a neck, and a trochanter which is the lateral large projection with a large crest. Muscular lines are present on the cranial and caudal surfaces of the femoral shaft. The distal end (epiphysis) of the femur has two condyles which articulate with the tibiotarsus bone medially, and the fibula laterally, and two epicondyles for muscular attachment. The two condyles are separated by the large intercondylar groove to separate the patellar sesamoid bone (knee bone) (Figure 2.14).

2.15.2 Patella

The patella is the sesamoid bone of the femorotibial muscles. It is covered by hyaline cartilage caudally, while the cranial surface has a groove for the passage of the ambiens muscle (König et al. 2016, pp. 65–66). The patella is an immovable lever against the lower leg of the bird (Koch 1973, p. 32).

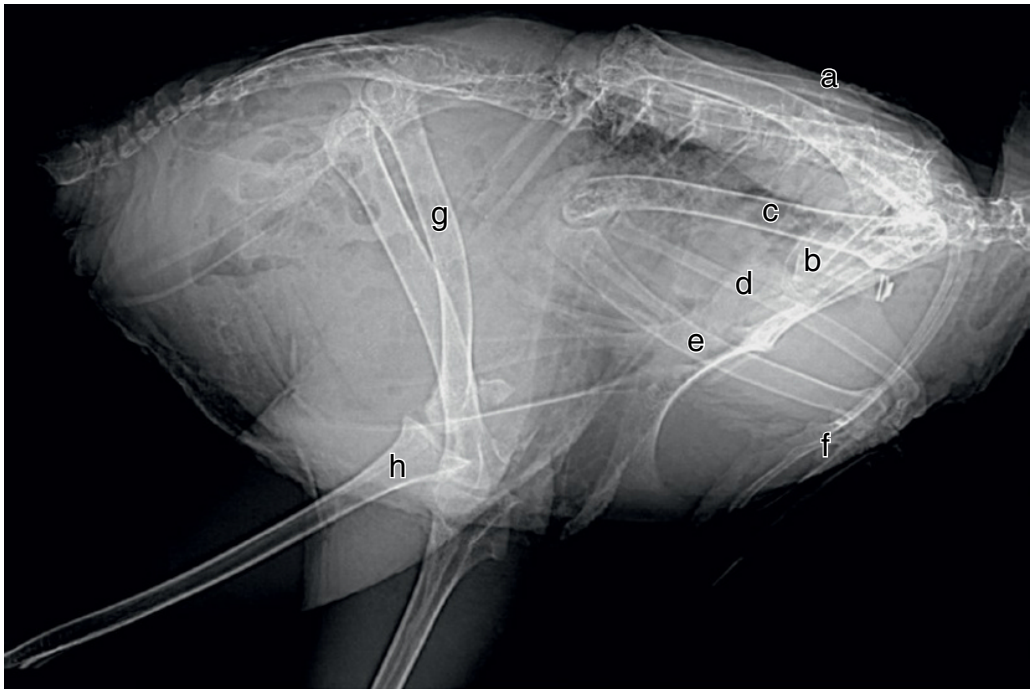

Figure 2.13 A chicken radiograph showing the (a) scapula, (b) coracoid, (c) humerus, (d) radius, (e) ulna, (f) metacarpal bones, (g) femur, and (h) tibiotarsus.

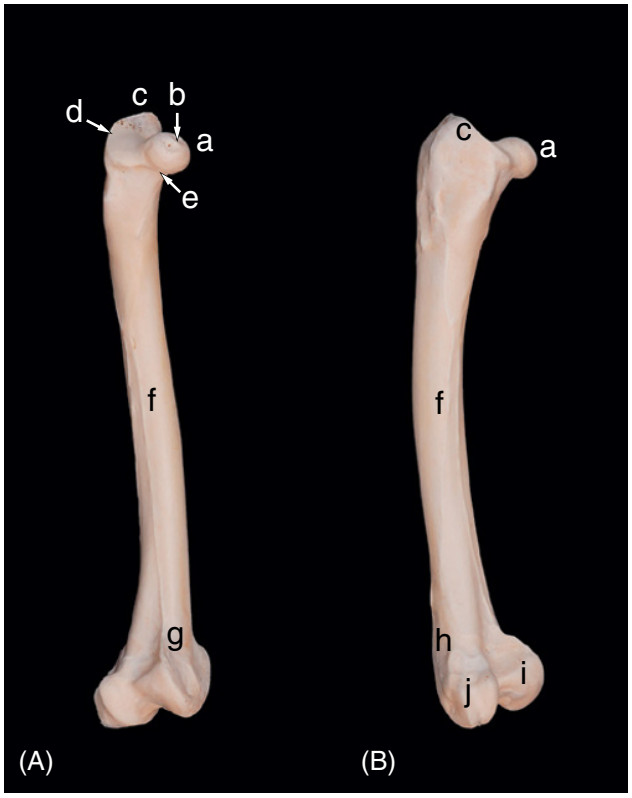

Figure 2.14 Left femur of chicken (A) caudomedial view and (B) caudolateral view, (a) caput (head), (b) fovea capitis, (c) trochanter, (d) trochanteric crest, (e) neck, (f) femoral shaft, (g) medial epicondyle, (h) lateral epicondyle, (i) medial condyle, (j) lateral condyle.

The frequent occurrence of calcification or ossification of the pelvic limb tendons would suggest that these tendons may be subjected to considerable stress forces, particularly when landing from flight, in the bipedal posture of birds (Agabalyan et al. 2013). The proximal tibial secondary ossification center may be a remnent of a fusion of a pre-existing sesamoid to the tibia as a further strengthening device for the insertion of the quadriceps femoris muscle tendon.

2.15.3 Tibiotarsus

The tibiotarsus bone is the largest and longest of the pelvic limb as it carries the entire bird body. It consists of the tibia (shin bone), the fibula, and the tarsal bones. Proximally, this bone has two condyles that articulate with the femur and carries a large crest proximally (cranial cnemial) and a lateral smaller crest (lateral cnemial) (Figure 2.15). The crest gives origin to the extensor muscles and the straight patellar ligament (Koch 1973, p. 32). The bony spur is present on the inner surface of the shaft approximately two thirds distally along the shaft. The fibula attaches to the upper lateral shaft of the tibiotarsus bone, ending approximately two thirds distally, on the inner surface of the shaft, in a bony spur. The distal extremity of the tibiotarsus has two prominent condyles for the articulation with the tarsometatarsus. Distally, the bone has three large trochleae for the articulation with digits II, III, and IV.

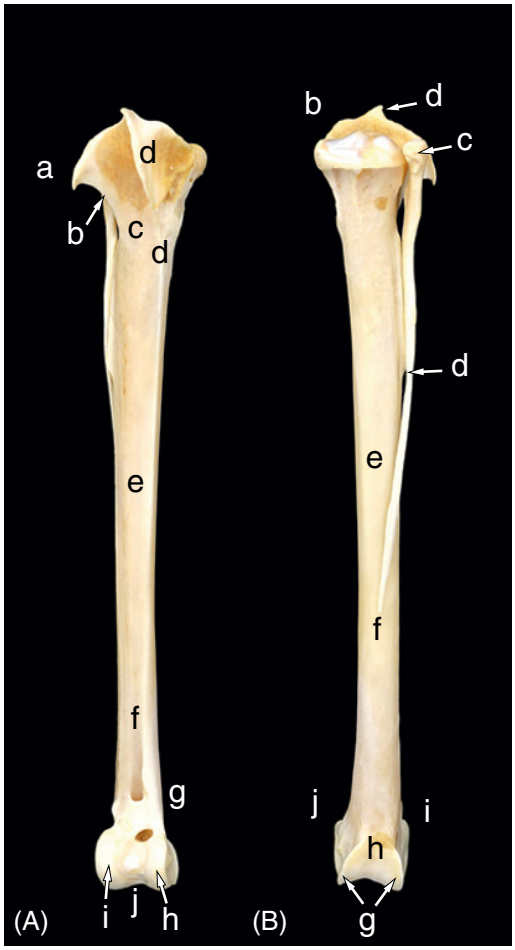

Figure 2.15 Chicken right tibiotarsus and fibula (A) cranial view, (a) patellar crest, (b) lateral cnemial crest, (c) intercnemial groove, (d) cranial cnemial crest (e) shaft of tibia, (f) extensor groove, (g) supratendinal bridge, (h) medial condyle, (i) lateral condyle, (j) intercondylar incisure and (B) caudal view, (a) tibial crest, (b) proximal condyles of the tibia, (c) caput (head) of the fibula, (d) shaft of the fibula, (f) spine of the fibula, (g) distal sagittal ridges, (h) trochlea, (i) lateral epicondyle, and (j) medial epicondyle.

2.15.4 Fibula

The fibula is greatly reduced in the chicken (Getty 1975, p. 1798). It has a head (caput), shaft (diaphysis), and a thin spine which ends at the lower one third of the tibiotarsus bone. It is intimately fused to the tibia at several places and is always shorter than the tibia (Figure 2.15). Therefore, it is an immovable bone.

2.15.5 Tarsometatarsus

The tarsometatarsus is formed by the fusion of the central tarsal and the distal tarsal with the II–IV metatarsal bones (Figure 2.1). There are four metatarsal bones in the bird, from which II, III, and IV are fused with the tarsal bones and with each other to form a single tarsometatarsus bone (the running bone). Metatarsal I is rudimentary and is separated from the rest of the other bones (Koch 1973, p. 38). The proximal end of the metatarsus has two protrusions directed caudally for the insertion of the Achilles tendon (common calcanean tendon). In mammals, this large tendon is inserted into the tuberosity of the calcaneus bone (Koch 1973, p. 37).

2.15.6 Digits

There are three digits (II, III, and IV) directed cranially opposing the first digit which extends caudally (Figures 2.1 and 2.2). The three digits II, III, and IV carry three, four, and five phalanges, respectively (Getty 1975, p. 1800). The terminal phalanx is claw-shaped (Koch 1973, p. 38).

2.16 Arthrosis (Joints)

The joints, in general, can be classified as immovable, movable, and mixed. An immovable joint is also called a synarthrosis which is an articulation with connecting tissue. Good examples of this kind of joints are the sutures between the skull flat bones (Chamberlain 1943, p. 17). A movable (diarthrodial or synovial) joint is either simple or compound having hyaline cartilage that covers the articular surfaces. These joints have ligaments which attach to the nearby ends of the articulating bones. A fibrous joint is less movable than arthrodial (gliding) joint. An example for this type of joint is the articulation between the coracoid bone with the superior end of the furcula (clavicle) by fibrous cartilage (Kaupp 1917, p. 44). A meniscus is another example of fibrous cartilage between the femur and the tibiotarsus which has elasticity and facilitates free movement of the joint. Fibrocartilages are also present in the skull and pelvis, resulting in the formation of a synchondrosis joint (Kaupp 1917, p. 56).

2.16.1 Joint Movements

There are several types of joint movements. Gliding is the simplest kind of motion without any angular or rotary movement. Angular movement usually presents between long bones by either increasing or decreasing the angle between the two articulating bones. Angular movements can be cranial or caudal, like flexion and extension, or medial or lateral.

Pulling the part toward the midline of the animal body is adduction while a motion taking the part away from the midline is abduction. Circumduction is a limited degree of motion between the head of one bone and the articular cavity of the other bone like the one between the humerus

and the scapula or the hip joints. Rotation is the movement of the bone upon an axis like the articulation between the atlas and the axis or the rotation of the radius upon the humerus. Pronation is the distal extremity of the radius that passes cranial to the ulna, thus rotating the hand inward. Rotation of the hand outward is supination (Kaupp 1917, pp. 56–57).

2.16.2 List of Joints

Classifications of forelimb and hindlimb type of joints listed below are mostly taken from Chamberlain (1943, pp. 17–21; Nickel et al. 1977, pp. 13–16).

2.16.2.1 Pectoral Limb Joints

Sternoclavicular is ligamentous synarthrosis, and syndesmosis.

Sternocoracoid joint is a hinge and gliding (diarthrosis, ginglymus, and arthrodial).

Coracoscapular joint (sutural) is synarthrosis, syndesmosis.

Coraco- and scapulaoclavicular joints are sutural joints (synarthrosis, syndesmosis).

Shoulder joint (coraco-scapulohumeral) is a ball and socket (diarthrosis, enarthrosis).

Elbow joint (humeroradial and ulnar) is a hinge joint (diarthrosis, ginglymus, and cochlear).

Proximal radioulnar joint is gliding (diarthrosis, arthrodial) which is continuous with the elbow joint.

Distal radioulnar joint is a fibrous (synarthrosis, syndesmosis).

Wrist joint is a hinge and gliding (diarthrosis, ginglymus, and arthrodial).

Carpometacarpal phalangeal joint is gliding (diarthrosis, arthrodial).

Interphalangeal joint is a hinge joint (diarthrosis, ginglymus with limited movements).

2.16.2.2 Pelvic Limb Joints

Synsacro and costocoxal articulations are gliding (diarthrodial, arthrodial) and ligamentous (synarthrosis, arthrosis, and syndesmosis).

Coxofemoral joint is a ball and socket (diarthrosis, enarthrosis).

Femorotibial joint is a hinge Joint (diarthrodial, ginglymus). It has two fibrocartilaginous menisci. The large amount of hyaluronate in the chicken meniscus, far more than necessary for proteoglycan aggregation, might be a particular characteristic of the avian tissue or might suggest that hyaluronate forms a gel embedding the collagen fibrils. The presence of dermatan sulfate in the chicken meniscus most probably indicates the existence of a second proteoglycan population (Pedrini-Mille et al. 1988).

Hock Joint is a hinge joint (diarthrosis, ginglymus).

Intertarsometatarsal joint is a gliding (diarthrosis, arthrodial).

Tarsometatarsal joint is a hinge (diarthrodial, arthrodial).

Metatarsophalangeal is a hinge joint (diarthrodial, ginglymus).

Tibiofibular joint is a ligamentous joint (synarthrosis, syndesmosis).

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3

Muscular System

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3.1 Introduction

The chicken body has smooth, cardiac, and skeletal muscles. They are all similar in their embryonic origin because they all develop from the mesoderm. The smooth muscles present within the wall of the visceral organs as well as the cardiac muscle in the heart develop from the splanchnic mesoderm, while the skeletal muscles develop from the somitic mesoderm.

Both smooth and cardiac muscles are involuntary and thus innervated by the autonomic nervous system. The avian somitic muscles are modified to help with flight. All skeletal muscles are innervated by the spinal nerves except for the muscles on the head which are innervated by certain cranial nerves. Smooth muscles are present in the wall of blood vessels and all tubular organs. Cardiac muscle by definition is only present within the heart. A small number of cardiomyocytes populate the base of the large blood vessels (aorta and pulmonary trunk).

Muscles on the head and neck plus the dorsal body region have less clinical significance; therefore, they will be listed but not discussed in detail. The emphasis in this chapter will be on the fore- and hind limbs and abdominal and pectoral regions.

3.2 Muscle Histology

Avian muscles are very similar to those of mammals. The muscle cell is called a myocyte (myofiber) and the position of the nuclei within the cells helps to differentiate their type. Skeletal muscle nuclei are at the periphery of the cell right under the plasma membrane (plasmalemma), while smooth and cardiac muscle cells have their nuclei in the center of the cells.

3.2.1 Smooth Muscle

Smooth muscle is involuntary and non-striated. The myofiber has the nucleus in the center of the cell like the cardiac myofiber (cardiomyocyte). Smooth muscles are present in the tunica media of the blood vessels and the muscular tunic of the tubular organs (Figures 3.1 and 3.2). It has slow contraction and requires less energy but can maintain contraction for longer compared to the skeletal muscle.

3.2.2 Cardiac Muscle

Cardiac muscle, as the name indicates, is present within the myocardium (atrial and ventricular walls) and the base of large blood vessels that originate from the heart (aorta and the pulmonary trunk). It is striated like skeletal muscle but involuntary. Thus, it is supplied by the autonomic nervous system (both sympathetic and parasympathetic). The cardiac myocyte has a central nucleus like the smooth muscle cell (Figure 3.3). Characteristic features of the cardiac muscle are the branching of the cardiomyocytes and the presence of the intercalated disks.

3.2.3 Skeletal Muscle

Muscles on the animal body, in general, account for about half of the weight of an animal, though the proportion varies with species, breed, age, sex, and method of husbandry. The muscle consists of myocytes (immature cells, myoblasts) grouped together to form a tube-like structure within the muscle (Dyce et al. 2010, p. 23). Myocytes and myofibers are used interchangeably and organized in a specific way within the muscle. Muscle contraction (shortening of the myotube) is a result of the shortening of the length of the aggregated myofibers within the muscle.

Anatomy and Histology of the Domestic Chicken, First Edition. Edited by Wael Khamas and Josep Rutllant.

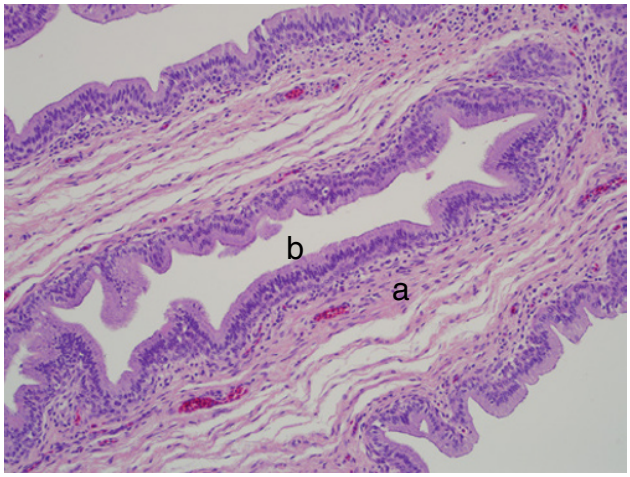

Figure 3.1 The inner cloacal surface of a chicken shows (a) smooth muscles within the wall of the cloaca and (b) simple columnar epithelium. H&E stain.

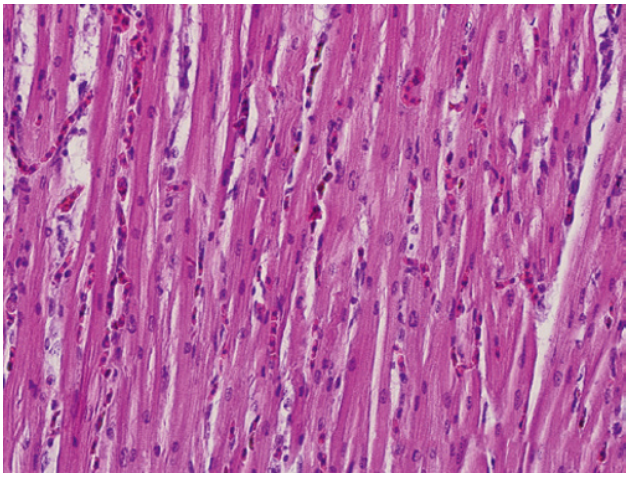

Figure 3.3 The longitudinal section of chicken cardiac muscle shows striations centrally located nuclei, and the branching of cardiocytes. H&E stain.

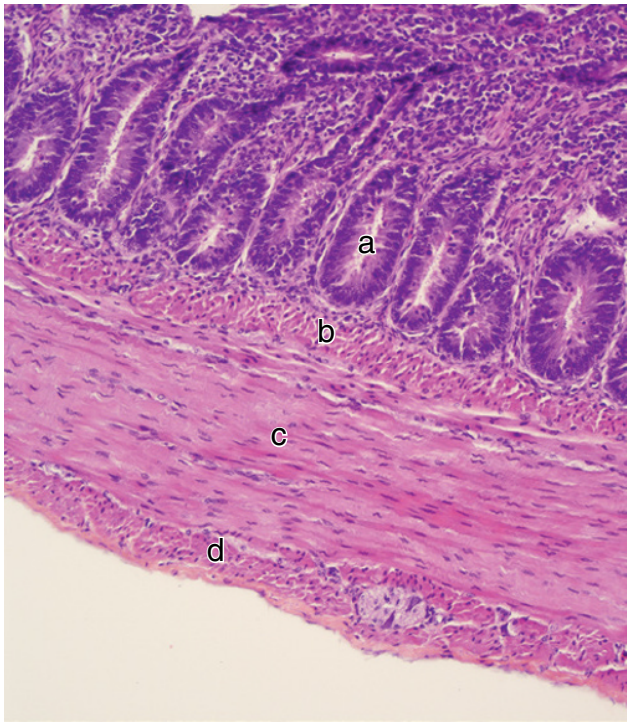

Figure 3.2 A section in the wall of the chicken intestine shows the three layers of smooth muscles: (a) glandular region within the tunica mucosa, (b) lamina muscularis, (c) inner circular, and (d) outer longitudinal muscles. H&E stain.

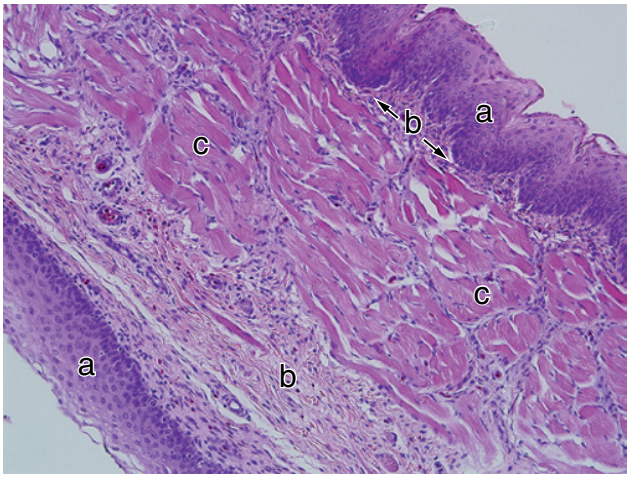

Figure 3.4 A section in the outer region of the cloacal opening shows (a) non keratinized stratified squamous epithelium, (b) propria submucosa, and (c) skeletal muscles. H&E stain.

The cross-sectional area of a muscle dictates the strength of the action (Figures 3.4 and 3.5).

A single skeletal muscle cell is called a myocyte or myofiber while a group of myofibers are called a fascicle (fasciculus pl. fasciculi). Bundles of fascicles together form the muscle. The connective tissue within the animal body forms the stroma within every organ and envelops the organs and structures. The connective tissue protects the organ and creates structural support by forming a capsule of varying thickness around organs. It extends inside the organ separating lobes and lobules from each other and anchors the epithelium to underlying tissues. It also functions as a route, to and from different organs or tissues, for blood vessels, lymphatics, and nerves. An individual myofiber within the skeletal muscle is surrounded by a very thin connective tissue layer composed mainly of collagen fibers (endomysium) with few reticular fibers. Fascicles of myofibers are separated by connective tissue composed of collagen with few elastic fibers (perimysium). The same composition is true in respect to the type of connective tissue that surrounds

Figure 3.5 A cross section of the thigh red muscle of adult chicken. Notice the connective tissue covering the entire muscle (a) epimysium, while the thinner tissue between fascicles of muscles is (b) perimysium. Individual myocytes are surrounded by the (c) endomysium. H&E stain.

bundles of fascicles (epimysium) (Figure 3.5) (Cross and Mercer 1993, p. 196). Each individual myofiber is surrounded by the sarcolemma (lemma, cell membrane, sarx means flesh thus muscle) and the cytoplasm is called sarcoplasm which contains cellular organelles.

A myofiber is composed of myofibrils. Myofibrils consist of myofilaments which are the smallest units of a myofiber. When evaluated with light and electron microscopy, skeletal muscle shows stripes with alternating light (I) isotropic and dark (A) anisotropic bands. The light band is bisected by a dark line (Z line). The distance between two Z lines is the sarcomere. During contraction of the muscle cell (myofiber), the distance between the two Z lines shortens.

3.3 Muscle Nomenclature

Muscles can be classified based on different criteria:

3.3.1 Structure and Arrangement of Fibers

Muscles can be classified as unipennate, bipennate, circumpennate, and multipennate. Unipennate muscles are muscles whose fibers attach to one side of the tendon. Bipennate muscles have two attachment sites, on the opposite side of a central tendon. When muscle fibers are arranged in a circular pattern, it is called circumpennate. Multipennate muscles are muscles with the fibers in multiple rows with the central tendon branching into two or more tendons. Most of the muscles of the pelvic limb of chicken can be classified as unipennate with long cord-like tendons. This arrangement permits their heavy bellies to be placed close to the bird's body.

3.3.2 Number of Heads

Muscles can be classified according to the number of heads like biceps, triceps, and quadriceps.

3.3.3 Action

A muscle can be described according to their functional action as flexor when the muscle decreases the angle of a joint, while extensor is used when a muscle acts to increase the angle of a joint. A muscle that brings part of the body closer to the midline is an adductor, while a muscle that moves the part away from the median plane is an abductor. A muscle that rotates part of the body forward and inward is a pronator while one that rotates the part backward and outward is a supinator. Other muscle names are dilator, levator, depressor, sphincter and tensor.

3.3.4 Shape/Morphology

A muscular belly can acquire different shapes that give the name of the muscle. Trapezius and rhomboideus are excellent examples. In general, muscles can have spindle, wide-flat, strap, or circular (orbicularis) shapes. Good examples of spindle shape are the muscles of the forelimb in mammals and the wing muscles in chicken. Elongated cord-like are those present on the vertebral column and pelvic limb. A flat sheet-like example is the body wall, specifically the abdominal muscles. Massive groups of muscles like the gluteal in mammals and the pectoral in birds are named by location. Circular muscles acting as sphincters are found in the vent (recto-coprodeal junction) of birds (Mahdi and McLelland 1989).

3.4 Types of Skeletal Muscle Fibers

Five different types of fibers are described in chicken, type I, IIA, IIB, IIIA, and IIIB (Billeter et al. 1992). Avian skeletal muscles comprise glycolytic (type II, fast-twitch) fibers and oxidative (type I, slow-twitch) fibers. However, compared with livestock, the skeletal muscles of poultry contain a relatively small number of oxidative fibers (Weng et al. 2022).

Using earlier nomenclature, skeletal muscle can be simply classified as red, white, or mixed depending on the amount of myoglobin pigments present within the cells. White muscle contracts quickly with more strength than red but is easily fatigued. Red muscle contracts slowly, contains myoglobin, generates ATP by oxidation of adipose tissue and carbohydrates, and therefore exhibits resistance to fatigue (Ashmore and Doerr 1971). However, this

classification does not adequately describe the variety of fiber types in chicken.

The pectoral muscles comprise primarily fast type IIB fibers which are primarily glycolytic. They are often referred to as "white muscles" because they are poorly vascularized with a very low concentration of myoglobin, a predominant contributor to meat pigmentation.

In contrast, muscles found on the thigh and leg of chickens (type I fibers) are "slow-twitch" aerobic fibers that are rich in mitochondria with a high concentration of myoglobin, hence the red color (Rosser and George 2011).

The breast muscles in chicken, used during flight, are white, fast contracting fibers for short and rapid bursts of activity. White muscle depends primarily on glycolysis to produce ATP and has little or no myoglobin. White muscle utilizes anaerobic metabolism for fast contraction but tires easily due to lactic acid buildup. Red muscle has more mitochondria, a higher content of lipid globules, and greater vascularity. The content of myoglobin dictates the degree of redness from low to high (Hodges 1974, p. 253). Examples of red muscle types in the chicken include those in the pelvic limb. The gastrocnemius muscle contains 10 times more myoglobin than the breast in chicken (O'Malley 2005, p. 108). The sartorius muscle in birds has mixed fiber types with a gradient of type I fibers from the red side of the muscle to the white aspect (Ashmore and Doerr 1971). Most avian muscles contain a mixture of red and white fibers; the proportion depends on how prolonged the activity of the muscle must be (King and McLelland 1984, p. 29). Other researchers studying fiber type in chicken skeletal muscles indicated that gross red coloration is not a useful diagnostic feature of slow muscles, since the deep adductor muscle, for example, is white. Fibers histochemically similar to mammalian type I (slow twitch) which occur in some of the avian muscles were investigated (Barnard et al. 1982).

3.5 Muscle Cells

3.5.1 Myocyte

Skeletal muscle cells (myocyte, myofiber) are long, cylindrical, multi-nucleated and striated. Skeletal muscle cells have high energy requirements, so they contain many mitochondria to generate sufficient ATP.

3.5.2 Satellite

The regenerative capability of skeletal muscle cells is variable based on embryonic origin of the tissue and its specific location in the body and function. It has limited regenerative capability due to the small number of satellite cells available. The satellite cell is established between the sarcolemma (plasmalemma) and the basement membrane. Satellite cells have been isolated and clonally separated from the pectoral muscle of the white leg horn chicken. These isolated cells can proliferate to result in the formation of a homogenous cell population. Several divisions take place before myotube formation is initiated (Yablonka-Reuveni et al. 1987). Therefore, many damaged myofibers will be replaced by connective tissue fibers from the epimysium, perimysium, and endomysium before the satellite cells replace a few of the damaged cells.

3.6 Skeletal Muscle Blood Supply

Arteries and veins enter and leave each muscle through the connective tissue fibers that form the epi-, peri-, and endomysium. These blood vessels arise or drain into larger blood vessels in the region.

3.7 Skeletal Muscle Innervation

Nerves run through the connective tissue around the muscle fibers like the blood vessels. Nerve fibers run obliquely between muscle fibers to be protected from damage during repetitive contraction and relaxation (Nickel et al. 1977, p. 215). Skeletal muscles of the trunk and limbs are innervated by the spinal nerves which carry motor fibers with their cell bodies in the ventral horn of the spinal cord. The axons of the ventral root (motor fibers) join the sensory fibers of the dorsal root as they exit through the intervertebral foramen. The sensory fibers arrive at the dorsal horn of the spinal cord through the dorsal root.

3.8 Muscle Spindle

A muscle spindle is composed of small myofibers (intrafusal fibers) surrounded by connective tissue within the muscle, while the rest of the functional muscle myofibers are considered extrafusal fibers. Intrafusal fibers lie parallel to the extrafusal fibers (Maier 1992). The number of muscle spindles varies depending on the muscle and are usually measured as the number of spindles per gram of muscle. Bird muscle spindle density is higher than mammals, and the muscles of the neck, and interosseous muscles are richly supplied with extrafusal myofibers (Adal and Chew Cheng 1980).

Golgi tendon organs are mechanoreceptors that monitor the contractile force produced by motor units. The motor unit is the functional unit of the skeletal muscle which is composed of one motor neuron and all myocytes

associated with it. Receptors in the Golgi tendon organs are most responsive to contractions by adjacent extrafusal muscle fibers. Afferent input from tendon organs is an unbiased measure of the contractile activity of the extrafusal fiber population of the muscle portion. In mixed muscle regions, slow-twitch fibers and fast-twitch fibers attach on given tendon organs, enabling them to monitor forces produced by slow and fast motor units. Most tendon organs are situated in mixed extrafusal fiber fields with high fast-twitch fiber content, indicating that in chicken leg muscles, sensory feedback from tendon organs is from fast motor units (Maier 1999).

3.9 Tendon

3.9.1 Histology

Muscles are indirectly inserted onto the bone through tendons. Individual myofibers are small and soft because of the nature of the function they perform. Contraction of a muscle may result in easy rupture of the muscle fibers if directly inserted on the bones. Therefore, the connective tissue fibers of endomysium, perimysium, and epimysium merge at the end of the fleshy portion of the muscle to form a tendon. The great tensile strength of the tendons is reflected in their structure. The tendon is the passive part of the muscle while the muscle is the active part which initiates contraction and relaxation.

The tendon is devoid of myofibers, and muscle cells are replaced by fibroblasts (tenocytes) which produce connective tissue (collagen fibers). The collagen fibers aggregate in bundles and run parallel to each other and with the direction of the muscle fibers (myofibers). For a variable distance before the tendon attaches to the bone, fibrocartilage replaces part of the terminal portion of the tendon to secure a better anchoring point of the tendon to the periosteum of the bone. Tendons are composed of individual collagen fibers, bundles (fascicles), and tendon parenchyma. Each of these parts is surrounded by a layer of connective tissue similar to the muscle. These connective tissues are the endotenon (endotendineum), peritenon (peritendineum), and epitenon (epitendineum) (Eurell and Frappier 2006, p. 42). Tendon collagen fibers blend with those of the bone periosteum and some fibers penetrate the outer osseous layer as Sharpey's fibers. The osteotendinous junction (enthesis) may be dense fibrous connective tissue, fibrocartilage or partially ossified depending on the size of the muscle and its function (Nickel et al. 1977, p. 26).

In birds, certain long tendons, especially those that continue from a large muscle (crossing more than one joint) have additional sheet-like structure with synovial membrane lining. The synovial membrane is lined by mesothelial cells on both sides with synovial fluid between the two layers. These two layers are parietal and visceral sheaths. The visceral layer is intimately attached to the epitendineum while the parietal joins the surrounding connective tissue. This allows it to be closer to the blood vessels and interstitial fluid. The connection between the parietal and visceral layers is the mesotenon (Nickel et al. 1977, p. 219). There are two cell types lining the synovial membrane around the tendon like the lining epithelium of any synovial joint cavity. These two cells are macrophage-like cells, which are simple cuboidal in shape, and fibroblast-like cells that are simple squamous flattened cells. The function of the macrophage-like cells is to phagocytize any debris or remnant of dead cells while the fibroblast-like cells secrete and absorb synovial fluid thus regulating the volume within the synovial space under normal physiological conditions (Kierzenbaum 2007, p. 162).

3.9.2 Tendon Modifications

Whether the muscle ends as an aponeurotic sheet (thin connective tissue extension of the muscle) or a tendon, the collagen fibers run in the direction of tension of the original muscle they extend from. Stress on tendons by use and growth of the muscle over time causes specific regions to incur tenocyte metaplasia, in which the tenocytes are transformed into chondrocytes. If severe stress is put on the tendon, the chondrocytes will undergo metaplastic changes and ultimately become osteoblasts. Tendons that have undergone metaplastic change are more resistant to stress than those that have not. Muscles that are very active (under high stress), such as those of the wing and the pelvic limb, typically have tendons that become ossified. Ossified tendons start to appear on radiographs after a certain age of the bird (as early as 15–20 weeks of age). In the hind limb, the flexor muscles start ossification earlier than the extensors. Also, the flexor muscles of the hind limb start ossification before the flexor muscles of the wings (Abdalla 1979).

3.10 General Description of Skeletal Muscle

The skeletal muscle consists of a head (caput) usually located at the origin of the muscle which is mostly proximal and less or non-movable in relation to other parts of the muscle. The belly (venter) is the middle of the muscle and usually the largest in diameter relative to other parts. The tail (cauda) is at the insertion site which is most of the time distally located and more movable relative to other parts.

Muscles that join the tendon with an angle tend to be strong in relation to their bulk. Muscle contraction expends more energy for action compared to tendons. Therefore, the arrangement of long tendons crossing over several joints in bird pelvic limb results in conservation of energy when movement is required.

3.11 Cutaneous Muscles

Cutaneous muscles in birds are very thin and variable in their distributions between different body regions. In the region of the patagium (see Chapter 1, External Features), they are extensions of the close by muscles like trapezius, serratus superficialis, pectoralis, biceps brachii, latissimus dorsi, and deltoideus. These muscles facilitate the spreading of the patagium during flight (Koch 1973, p. 47). Other muscles are also listed as cutaneous in avian; cutaneous colli lateralis, cutaneous nuchalis, cutaneous cleidodorsal, cutaneus spinalis dorsal and cutaneous aucheniatria, cleidotrachealis, cleidoventralis, trunciventralis, metapatagialis, cutaneous iliacus, and costohumeralis (Chamberlain 1943, pp. 25–26).

3.11.1 Bird Muscles

In flying birds, the main and the largest muscles are found in the pectoral region. However, in non-flying birds, the ratio of muscle relative to body weight is a little less than in flying birds. The concentration of muscles on the ventral portion of the body, close to the center of gravity, provides stability for flight (O'Malley 2005, p. 107).

3.12 Flight Muscles

3.12.1 Pectoral

Muscles of flight are pectoral (Figure 3.6) and supracoracoid. The pectoral provides the powerful wing down stroke, while the supracoracoid provides the upstroke (Raikow 1985). The muscles of the breast and pectoral region are taken from Chamberlain (1943, pp. 30–31) and Proctor and Lynch (1993, pp. 150–173). The sternum carina may support the pectoral muscle to avoid exerting pressure on the supracoracoid muscle while contracting (Pennycuick 1972, p. 22; King and McLelland 1984, p. 30). The large muscles of the pectoral region may be divided

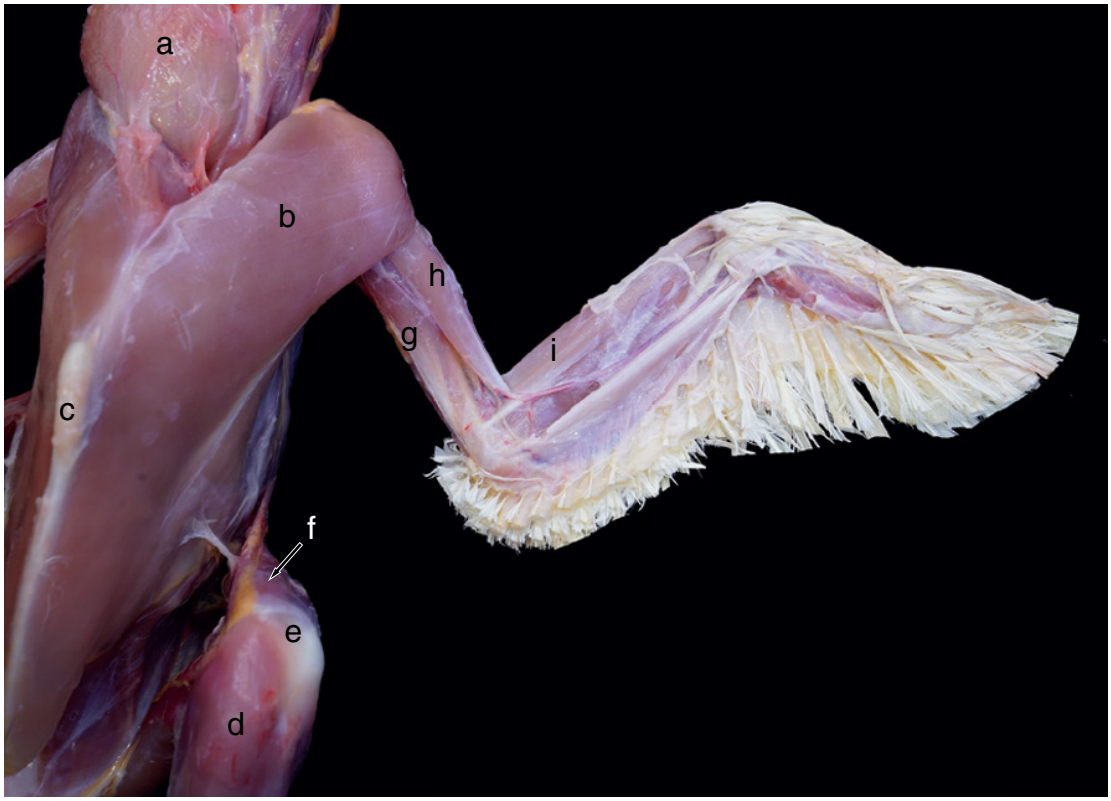

Figure 3.6 Dissected chicken, ventral view. (a) Crop (ingluvies), (b) major pectoral m., (c) sternum (keel), (d) fibularis longus m., (e) knee, (f) cranial iliotibial (sartorius) m., (g) triceps brachialis m., (h) biceps brachialis and (i) extensor carpi radialis m.

into superficial (pectoralis major) and deep (minor or subclavius). The major pectoral muscle originates from the carina of the sternum, furcula, and sternal ribs. It is inserted on the lateral tuberosity and crest of the humerus. The pectoral muscle mass is routinely used to assess the bird's health condition and may also be used for intramuscular injection of medications. Though the sternum is very close and could be easily punctured. Therefore, if injection is required in the pectoral muscles, the needle needs to be parallel to the sternum to avoid puncturing the birds' large size liver (O'Malley 2005, p. 109). Supracoracoid muscle (pectoralis minor, subclavius) attaches to the carina of the ventral sternum beneath the pectoral muscles (Proctor and Lynch 1993, p. 156). The tendon of the supracoracoid muscle passes through the triosseal canal to insert on the dorsal tubercle of the humerus. It elevates the wing for flight. It is about one-fifth the size of the pectoralis and is the primary wing elevator, which is active during upstroke, particularly at slow to moderate speeds and during hovering. At faster flight speeds, wing elevation is produced, and lift is maintained, passively by aerodynamic forces acting on the wings, which remain extended during the upstroke (Rayner 1988; Tobalske et al. 2003).

3.12.2 Wing Muscles

Wing muscles are strong and highly specialized with short bellies and long tendons. They are rarely compared to the corresponding muscles of mammals. The dorsal group of muscles are thin and play a role in fixing the wing to the bird body and thus powering flight (Koch 1973, pp. 51 and 154) (Figures 3.6–3.8).

3.12.2.1 Ventral Wing Muscles

3.12.2.1.1 Triceps Brachii The triceps brachii muscle acts to flex the shoulder joint and extend the elbow. It has two separate heads: the long arises from the scapular neck just posterior to the glenoid fossa, the humeral arises from the inner surface of the head and medial tuberosity of the proximal end of the humerus (Figures 3.9 and 3.10). All heads insert on the olecranon process of the proximal ulna. It receives blood from the deep brachial artery and innervation from the radial nerve (Proctor and Lynch 1993, p. 158; Nickel et al. 1977, p. 33).

3.12.2.1.2 Biceps Brachii Biceps brachii is a flexor muscle of the wing (elbow) just ventral to the humerus along its length. It also assists in the extension of the shoulder. Two heads are identified, one originates from the clavicle, coracoid and the other from the proximal end of the humerus. Its two tendons insert on the proximal end of the radius and ulna (Koch 1973, p. 52). Biceps brachii innervation is by the medio-ulnar (inferior brachial) nerve and the blood supply by the brachial artery.

3.12.2.2 Dorsal Wing Muscles

3.12.2.2.1 Deltoid The deltoid muscle covers the dorsal area of the shoulder and acts to pull the wing up and backward, thus flexing the shoulder joint. It is too small to raise the wing alone, therefore it works with other muscles. It originates from the scapula, clavicle, and coracoid to be inserted on the deltoid tuberosity of the humerus (Figure 3.11). It is supplied with blood by the circumflex humeral and axillary arteries. The subscapular and axillary nerves sends branches to innervate the muscle (Proctor and Lynch 1993, p. 162).

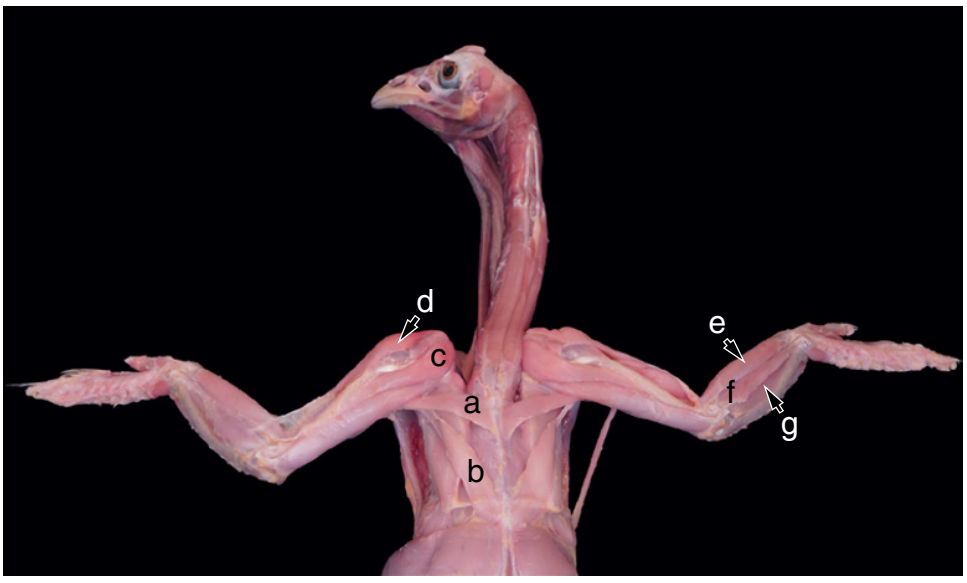

Figure 3.7 Dissected dorsal view of the muscles of the back of the chicken. (a) and (b) Latissimus dorsi m., (c) and (d) deltoid m., (e) extensor carpi radialis m., (f) common digital extensor m., and (g) extensor carpi ulnaris m.

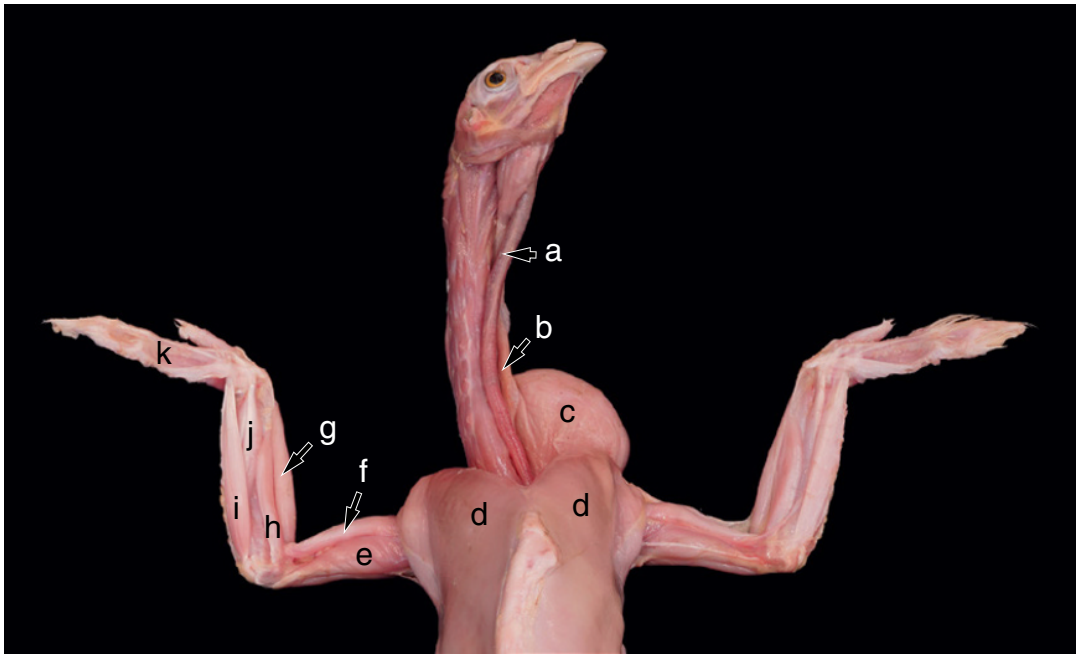

Figure 3.8 Dissected chicken, ventral view. (a) Trachea, (b) esophagus, (c) crop, (d) major pectoral m., (e) triceps brachialis m., (f) biceps brachialis m., (g) extensor carpi radialis m., (h) superficial pronator m., (i) flexor carpi ulnaris m., (j) deep digital flexor m., and (k) ventral interosseous m.

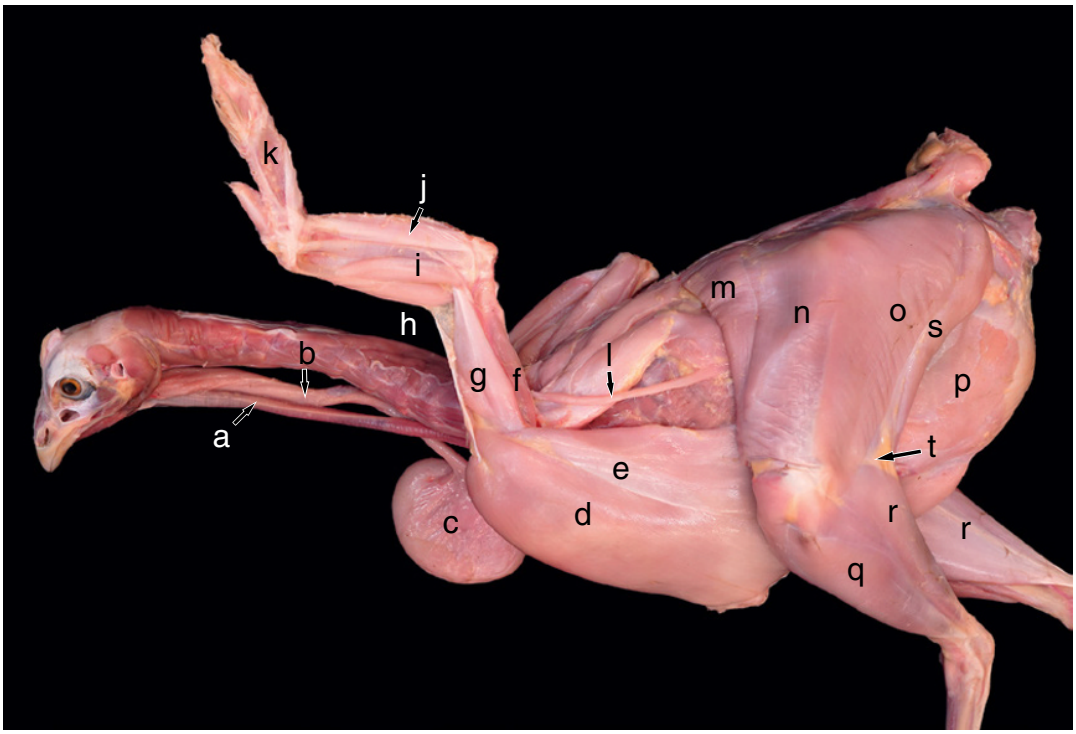

Figure 3.9 Dissected chicken left lateral view. (a) Trachea, (b) esophagus, (c) crop, (d) major pectoral muscle, (e) minor pectoral (supracoracoid) m., (f) triceps brachialis m., (g) biceps brachialis m., (h) extensor carpi radialis m., (i) superficial and deep pronators m., (j) flexor carpi ulnaris m., (k) ventral interosseous m., (l) cutaneous costohumeralis m., (m) cranial iliotibial (sartorius) m., (n) lateral iliotibial m., (o) caudal iliotibial m., (p) external abdominal oblique m., (q) long fibular m., (r) gastrocnemius m., (s) semimembranosus m., and (t) semitendinosus m.

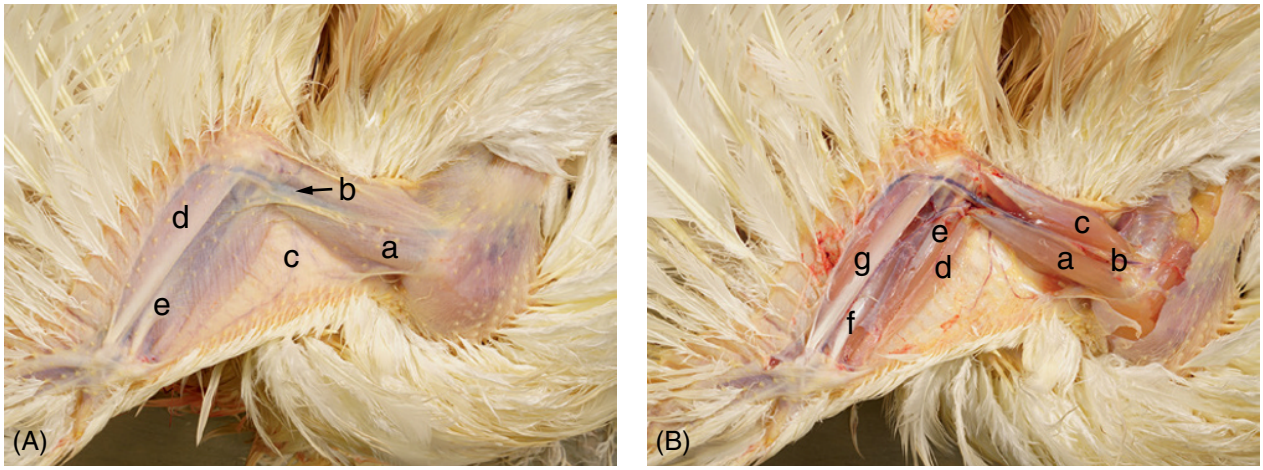

Figure 3.10 (A) Ventral aspect of the wing of adult rooster, feathers plugged and undissected. (a) Biceps brachii m., (b) basilic artery, (c) patagium, (d) flexor carpi ulnaris m., and (e) superficial pronator m. (B) Ventral aspect of the wing of adult rooster. (a and b) Biceps brachii m., (c) triceps brachii (cubiti) m., (d) extensor carpi (metacarpi) radialis m., (e) superficial pronator m., (f) deep digital flexor m., and (g) flexor carpi ulnaris m.

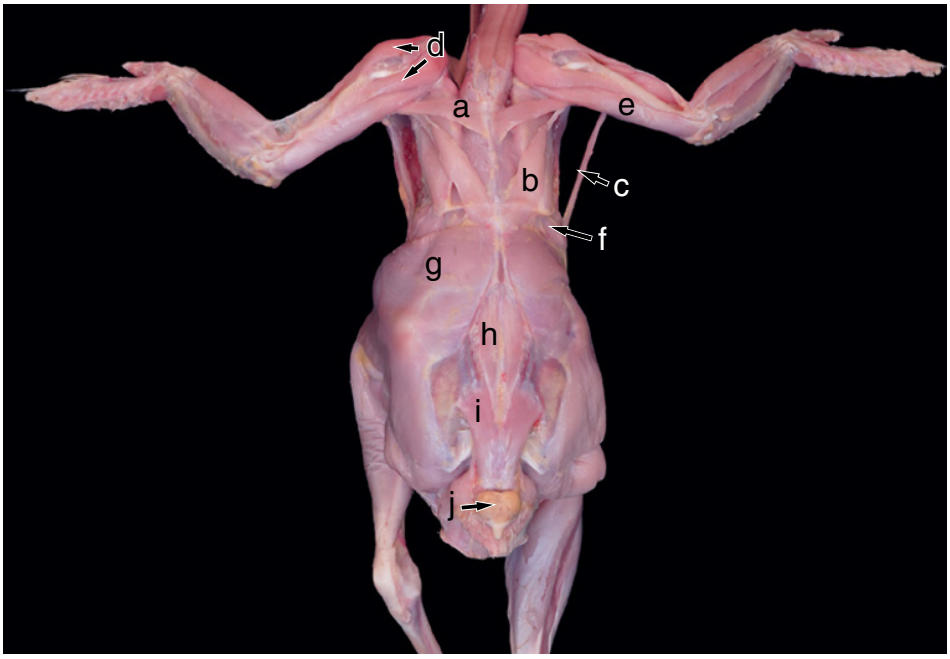

Figure 3.11 Dissected chicken, dorsal view. (a) and (b) latissimus dorsi m., (c) cutaneous costohumeralis m., (d) deltoid (propatagial part) m., (e) triceps brachialis m., (f) cranial iliotibial (sartorius) m., (g) lateral iliotibial m., (h) longissimus dorsi m., (i) levator coccygeus m., and (j) uropygial gland.

3.12.2.2.2 Latissimus Dorsi The latissimus dorsi muscle is reduced in birds because of the fusion of much of the vertebral column and no need for heavy thick muscles in the back. It has two heads (major and minor), and it flexes the shoulder joint, thus folding up the wing and carrying it during rest. This way, it adducts and flexes the arm moving it backward and dorsally. The heads originate from the dorsal midline at the level of the spinous processes of the two first and the two last thoracic vertebrae to insert on the crest of the lateral surface of the humerus (Figure 3.11 and 3.12). The dorsal thoracic artery supplies this muscle, and the branches of the dorsal brachial nerves innervate it.

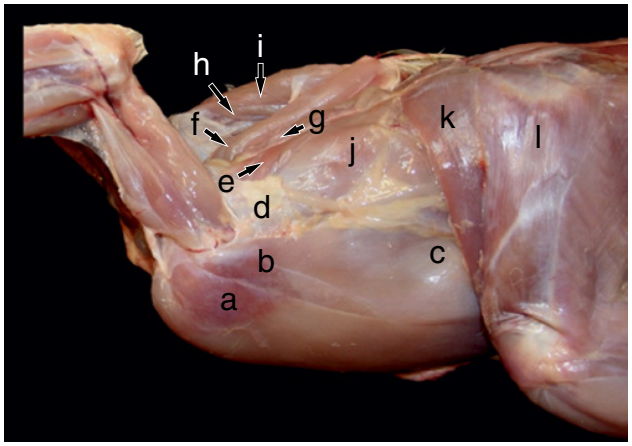

Figure 3.12 Lateral aspect of the left musculature of the adult rooster. (a) Superficial pectoral m. (pectoralis major, pars thoracis), (b) supracoracoideus m. (pectoralis minor), (c) external abdominal oblique m., (d) caudal lateral process of the sternum, (e) scapulohumeralis m., (f) teres major m., (g) caudal part of latissimus dorsi m., (h) rhomboid m., (i) trapezius m., (j) serratus ventralis m., (k) cranial iliotibial (sartorius) m., and (l) lateral iliotibial m.

3.12.2.2.3 Extensor Carpi Ulnaris Extensor carpi ulnaris muscle extends the manus region, thus moving the primary feathers attached to this region. The tendon is present at the anterior end of the wing and onto the carpometacarpal region and the phalanges of the wings. It draws the carpometacarpal from the body into a fully extended position. It extends the carpus. The ulnar artery brings the blood to it and the radial nerve controls its movement.

3.12.2.2.4 Extensor Carpi Radialis Extensor carpi radialis muscle is like the extensor carpi ulnaris muscle in respect to the origin, insertion, and function. The lateral epicondyle of the humerus is the origin and metacarpal bone is the insertion. The muscle acts to flex the elbow and extend the carpal joints. The cranial radial artery supplies it with blood and the radial nerve controls its movement.

3.12.2.2.5 Flexor Carpi Ulnaris Flexor carpi ulnaris is also called entepicondyloulnaris by Koch (1973, p. 54). The muscle is the main flexor muscle of the mid wing (carpal joint). The muscle belly can be found along the wing's trailing edge and onto the carpometacarpal region's surface. It pulls the outer wing and helps fold the wing into the resting position.

The tendon of this muscle contains a sesamoid bone for the passage over the olecranon process of the ulnar bone. Bands from this muscle tend to pass to the secondary flight feathers (Koch 1973, p. 54). It is supplied with blood by the interosseous artery while the ulnar nerve controls its movement (Nickel et al. 1977, p. 33).

3.13 Muscles of the Pelvic Region

The thigh muscles are dark in color and rich in sarcoplasmic reticulum, in contrast to the white muscles of the breast (pectoral muscles) (Figures 3.13 and 3.14).

3.13.1 Thigh Muscles

3.13.1.1 Iliotibialis Complex

This group of muscle consists of (i) cranial iliotibial (sartorius) which is a strap-like muscle like that of the mammals, (ii) lateral iliotibial, and (iii) caudal iliotibial muscles.

i) **Cranial iliotibial**
Cranial iliotibial muscle forms the craniolateral border of the thigh. It originates from the craniodorsal rim of the preacetabular iliac crest, dorsal fascia through aponeurotic attachment, median dorsal ridge of the synsacrum and preacetabular ilium. It inserts on the patella's medial surface, contributing to the patellar ligament's formation. This muscle acts to flex the hip and extend the knee and tibiotarsus joints. It is supplied by the femoral nerve and the femoral artery brings the blood supply.

ii) **Lateral iliotibial**
The lateral iliotibial muscle is a thin and triangular muscle on the lateral surface of the thigh. It originates from the iliac crest and inserts on the fascial sheath of the femorotibial muscle, and therefore, contributes to the patellar ligament formation. It assists in extension or flexion of the thigh (shank) with lateral rotation of the tibiotarsus.

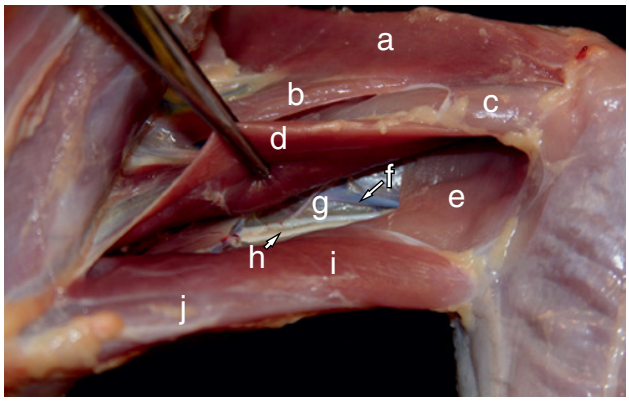

Figure 3.13 Medial aspect of the thigh of adult male chicken. (a) Cranial iliotibial (sartorius) m., (b) ambiens (pectineus) m., (c) internal femorotibial m., (d) puboischiofemoralis m., (e) accessory head of gastrocnemius m., (f) ischiatic (sciatic) vein, (g) ischiatic (sciatic) artery, (h) ischiatic (sciatic) nerve, (i) medial crural flexor m. (semimembranosus), and (j) lateral crural flexor m. (semitendinosus).

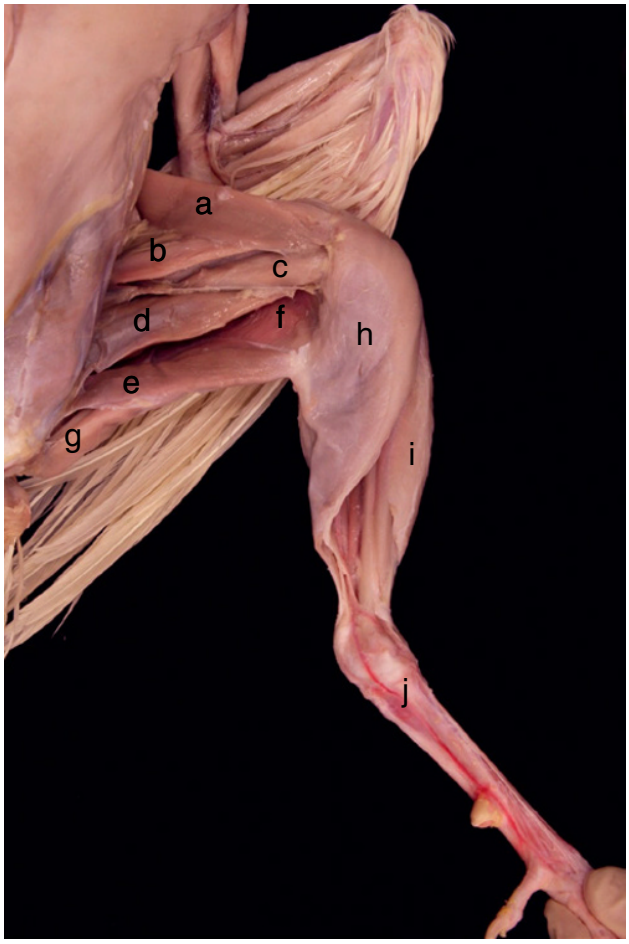

Figure 3.14 Medial aspect of the thigh of embalmed adult male chicken. (a) Iliotibialis cranialis m. (anterior) sartorius, (b) ambiens (pectineus) m., (c) internal femorotibial m., (d) puboischiofemoralis m., (e) semimembranosus (medial crural flexor) m., (f) distal portion of semitendinosus m., (g) semitendinosus (lateral crural flexor) m., (h) gastrocnemius medial head m., (i) fibularis longus m. (peroneal), and (j) common great metatarsal artery.

iii) **Caudal iliotibial, iliofibular muscle (biceps femoris)**
It lies deep to the lateral iliotibial muscle. It originates from the preacetabular iliac crest opposite to the acetabulum and inserts by a strong and rounded tendon on the proximal end of the fibular shaft between the external and medial heads of the gastrocnemius muscle. It flexes the knee, and it is supplied by the ischiatic nerve and the hypogastric artery (Getty 1975, p. 1829).

3.13.1.2 Iliacus Muscle

This muscle originates from the lateral surface and ventral edge of the ilium in the preacetabular region. It inserts on the proximal third of the medial surface of the femur on the trochanter minor. The muscle flexes the hip joint, and the femoral nerve supplies it with innervation. Its blood supply is from the spinal branches in the lumbar and sacral regions.

3.13.1.3 Adductor (Pubischiofemoralis Muscle)

The adductor muscle consists of two parts. It extends the hip joint and adducts the thigh. It originates from the ventral border of the pubis and ischium. It inserts on the caudodistal surface of the femur and its medial condyle. The obturator nerve brings its innervation from the lumbosacral plexus like mammals. Blood supply is from the femoral artery (Nickel et al. 1977, p. 37).

3.13.1.4 Semimembranosus Muscle

Another name for this muscle is medial crural flexor (Getty 1975, p. 1833). It is a thin flat muscle of the caudomedial surface of the thigh. It originates from the lateral and the ventrolateral surface of the ischium. It has a flattened tendon which passes between the medial and the lateral heads of the gastrocnemius muscle to insert on the caudal surface of the tibiotarsal region. It flexes the tibiotarsus joint. Innervation is through the ischiatic nerve and blood supply from the hypogastric artery (Proctor and Lynch 1993, p. 170).

3.13.1.5 Semitendinosus Muscle

Semitendinosus muscle is also named lateral crural flexor (Getty 1975, p. 1833). It arises from the postacetabular iliac crest and lateral surface of the ilium and ischium to insert by a tendon to the medial surface of the tibia. It extends the hip and flexes the knee. It is innervated by the ischiatic nerve while the hypogastric artery serves as the main blood source (Proctor and Lynch 1993, p. 170).

3.13.1.6 Quadratus Femoris Muscle

The quadratus femoris muscle lies just posterior to the sartorius muscle near the anterior border of the thigh (femur). It extends the hip joint. It originates from the lateral surface of the ilium, caudal to the ischiatic foramen. The muscle inserts on the caudal border of the femur. Innervation is through the femoral nerve and blood supply by the hypogastric artery (Proctor and Lynch 1993, p. 166; Nickel et al. 1977, p. 36).

3.13.1.7 Ambiens (Pectineus) Muscle

This muscle is homologous to the pectineus muscle in mammals. It is fusiform in shape tapering distally toward the tendon of insertion. Ambiens muscle originates from the pectineal process of the ilium cranioventral to the

acetabulum and the proximal part of the pubic bone to insert on the fascia above the proximal tibia at the tibial crest, thus contributing to the patellar ligament. It flexes the thigh. The femoral nerve from the lumbar plexus innervates the muscle and blood arrives to it through the femoral artery (Getty 1975, p. 1834).

3.13.2 Leg (Crural) Muscles

3.13.2.1 Anterior Tibial

The anterior tibial muscle covers the anterior surface of the leg deep to the long fibular muscle. It originates from the distal end of the femur and inserts on the proximal end of the tarsometatarsal region. It is a powerful flexor of the foot and the tarsometatarsal joint helping the bird to lift the foot off the ground. Proximally, it is separated into two heads while distally these two heads fuse together. It is innervated by the peroneal nerve and gets its blood supply from the anterior tibial artery (Proctor and Lynch 1993, pp. 164–166).

3.13.2.2 Gastrocnemius Muscle

The gastrocnemius is the largest crural muscle by mass and the strongest muscle which covers the most caudal surface of the leg. It has three heads, rarely two (medial, lateral, and proximal/tibial). Two heads originate from the femur while the third one from the tibia. It passes from the knee down to the common calcanean tendon (tendino-Achilles in mammals). The nomenclature of the three parts of the gastrocnemius is different in different textbooks (Getty 1975, p. 1838). It acts to extend the tarsometatarsal joint and plantarly flex the digits of the foot. The innervation of this muscle originates from the ischiatic nerve (tibial n.) and the blood supply is through the femoral artery and vein.

3.14 Abdominal Muscles

The abdominal muscles are very thin, and the portions of the muscle used for insertion are called aponeuroses. These muscles protect the visceral organs and assist in respiration (expiration) and defecation. The innervation of these muscles comes from several sources because of their extensive nature. They are all supplied by the thoracic (intercostal branches) and lumbar/sacral spinal nerves (Nickel et al. 1977, pp. 31–33). Intercostal arteries bring blood to these muscles.

3.14.1 External Abdominal Oblique

The external abdominal oblique muscle is the most superficial muscle under the hypodermis. It originates from the lateral surface of the ribs and their uncinate processes and an aponeurosis from the ilium and pubic bones. It inserts through a thin aponeurotic extension on the lateral border of the sternum to terminate on the linea alba. It acts to compress the abdominal wall and assist in expiration.

3.14.2 Internal Abdominal Oblique

The internal abdominal oblique muscle is the second layer of muscle under the hypodermis. It originates from the ventral border of the ilium and preacetabular border of the pubic bone. It inserts on the caudal border of the last vertebral rib and unites with the aponeurosis of the rectus abdominal muscle, thus contributing to the rectus sheath. It compresses the abdomen and brings about expiration.

3.14.3 Rectus Abdominal

The rectus abdominal muscle by definition is a straight muscle which originates from the lateral border of the sternum and caudal border of the last sternal rib. It inserts on the ventral border of the caudal half of the pubic bone. It functions in cooperation with other abdominal muscles to compress the body wall, assist in expiration, and elevate the sternum.

3.14.4 Transverse Abdominal

The transverse abdominal muscle acts as a belt on both sides of the abdominal wall. It originates from the ventral border of the pubic bone, preacetabular part of the ilium, and internal surface of the last three ribs. It inserts on the internal surface of the sternum and median union of the aponeurosis with that of the opposite muscle. It acts to contract the abdominal wall and assist in expiration.

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4

Digestive System

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4.1 Introduction

In this chapter, the digestive system of the chicken will be divided as: oral cavity and pharynx (beak, tongue, salivary glands, and taste buds), esophagus (pars cervicalis, crop, and pars thoracica), stomach (proventriculus, isthmus, and ventriculus), small intestine (duodenum, jejunum, and ileum), and large intestine (colorectum and ceca). The cloaca leads to the vent and is considered a continuation, or the end point, of the digestive system (Nickel et al. 1977, p. 56) (Figure 4.1). Liver and pancreas, as associated digestive organs, will also be included in this chapter.

4.2 Oral Cavity and Pharynx

In birds, the relationship of the oral (mouth), nasal and pharyngeal cavities is different than in mammals. Chicken, like most birds, lacks a soft palate and the oropharyngeal isthmus, thus forming a common space between oral and pharyngeal cavities (King and McLelland 1979, p. 71; Baumel et al. 1993, pp. 302–303). The oropharyngeal cavity communicates with the right and left halves of the nasal cavity through a slit-like opening located in the midline called the choana (Figure 4.2).

Although difficult to justify, and as an agreement, the caudal limit of the oral cavity of the chicken is at the level of the most caudal transverse row of papillae on the hard palate and the papillae on the base of the tongue (McLelland 1975, Chapter 63, Sisson) (Figure 4.2). The entrance to the oral cavity is characterized by the absence of lips and teeth. Instead, a hard keratinized epidermal structure named beak, bill, or rhamphotheca is present (see below). The roof of the oral cavity is the hard palate which has several visible ridges with caudally oriented papillae (Figure 4.2). The cheeks are very much reduced, and the floor of the mouth cavity has the median fold of the mucosal membrane connected to the free portion of the tongue and is called the lingual frenulum.

The pharynx is a thickened muscular tube that connects the oral cavity to the digestive (esophagus) and respiratory (nasal cavity and larynx) systems. In the dorsal aspect of the pharynx (roof), the opening of the infundibular slit is present (Figure 4.2j). It is the common opening of the right and left auditory tubes (Eustachian). Around the infundibular slit, there is a well-developed pharyngeal tonsil. The floor of the cranial portion of the pharynx is mostly formed by the root or base of the tongue.

As mentioned before, distributed over the palate, tongue, and pharynx, there are abundant modifications of the mucous membrane in the shape of ridges and papillae. The ridges, mainly distributed in the palatine region, are oriented longitudinally (lateral and median palatine ridges or swelling) and transversally (several rows of transversal palatine ridges) (Figure 4.2). There are clearly visible caudally directed papillae, highly keratinized, and conical in shape, arranged in different areas of the oropharynx. Dorsally in the oral cavity, the palatine papillae are associated with the transverse palatine ridges while the caudodorsal pharyngeal papillae are present around the infundibular slit (Figure 4.2c). At the floor of the oropharynx, separating the oral and pharyngeal cavities, a transverse row of lingual papillae is present at the base of the tongue. Caudal to those, there is a distinct row of conical papillae associated with the caudal border of the laryngeal mound, called the caudoventral pharyngeal papillae (Figure 4.2g).

The oral cavity and the pharynx are lined by a stratified squamous epithelium which can be keratinized in the areas more exposed to abrasion. In the chicken, keratinization is found in the caudally oriented papillae (Figure 4.3a) and at the apex and ventral surface of the tongue (Hodges 1974, pp. 6–38, 44). The area of the junction between the pharynx and the esophagus is lined by a thick

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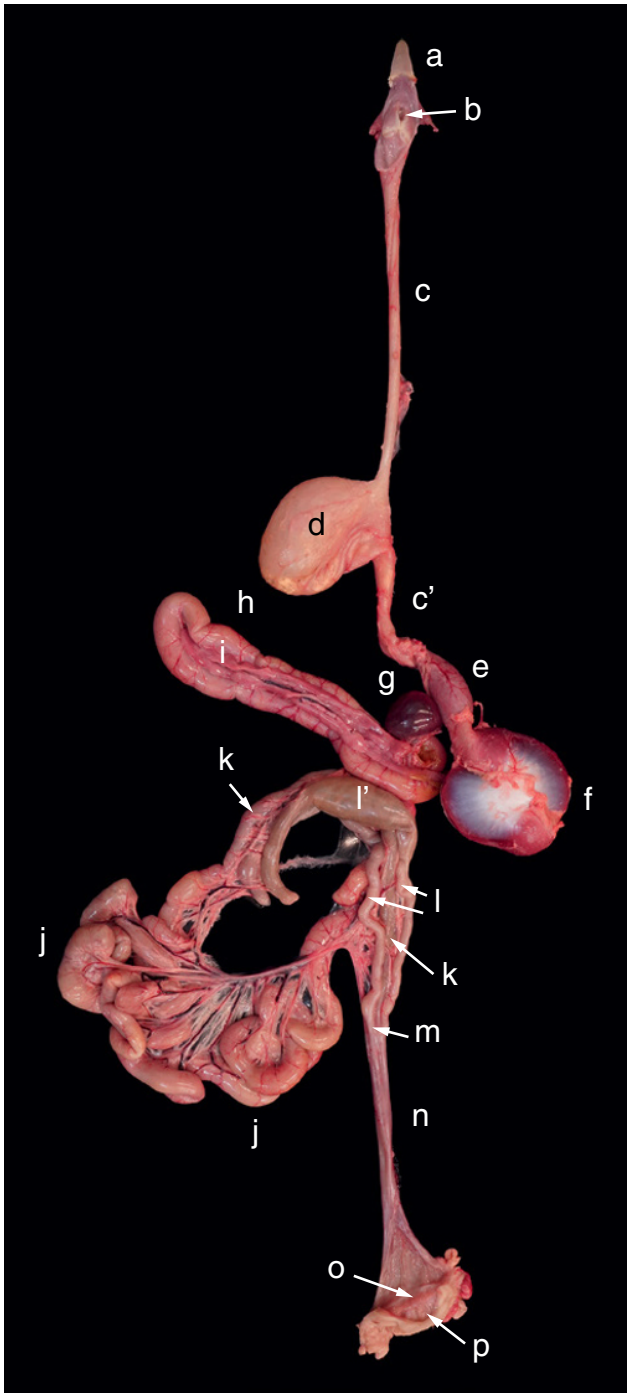

Figure 4.1 Dissected chicken digestive tract. Tongue (a), larynx (b), cervical, (c) and thoracic (c') portions of the esophagus, crop (d), proventriculus (e), gizzard (f), spleen (g), duodenum (h), pancreas (i), jejunum (j), ileum (k), ceca (l) and body of the cecum (l'), ileocecal junction (m), rectum (n), cloaca (open) (o), and vent (p).

highly keratinized stratified squamous epithelium with papillae. As the esophagus is approached, keratinization of the epithelium decreases, as well as the number of glands. A characteristic smooth muscle layer of the lamina muscularis mucosae starts to appear at this junction. Lymphoid follicles or tonsils are also present at this site.

4.2.1 Beak (Rhamphotheca)

The bill or beak is composed of the bones of the upper (superior, maxillary) and lower (inferior, mandibular) jaws and the highly keratinized structure (rhamphotheca) covering them (Figure 4.4). The shape of bird's beak is dictated genetically according to the need of the bird and usually follows the prehension method, and type of food (flesh, grain of any kind, sizes, etc.). The beak of the chicken is versatile and has pointed sharp edges. There are lots of functions listed for the beak, to mention few, eating, preening its own body or female head or feather arrangement, drinking, playing, grasping, mating, removing ectoparasites, and pecking in a fighting act (cannibalism) or for defense (Iqbal and Moss 2021). The beak is pointed at its tip and the upper and lower portions meet at the angle of the mouth. The base of the beak is sharply convex in the upper portion where it forms the operculum. The operculum covers the external nares in one-day-old chicks. The keratinized region of the beak is supported caudally by the jawbones. The outer dorsal midline surface of the beak is called the culmen and the ventral midline ridge of the lower part of the bill (gnathotheca) is called the gony. The cutting edges of the beak are called tomia (singular tomium). When the mouth is closed, the two edges of the beak, upper and lower, do not come together as the lower edges glide inside the edges of the upper beak.

A small protrusion is found in one-day-old chicks at the surface of the upper beak. It is used to break the shell of the egg during hatching and is usually shed soon after. This protrusion is commonly referred to as the egg tooth, although it is not related to true teeth. It is made of keratinocytes accumulating specific β-keratins (Shames et al. 1991; Alibardi 2014). Maturation of the egg tooth leads to its hardening which is completed before hatching. Later, the cornification of the rhamphotheca (beak) is completed and the egg tooth is lost (Wang et al. 2017).

The process of cutting the beak is called debeaking (or beak trimming since the whole beak is not removed) and, if done, is preferably performed on one-day-old chicks, as the beak is a highly innervated structure. One third of the distance from the tip of the beak is usually cut to prevent bleeding. Debeaking is routinely exercised in laying breeds to prevent cannibalism and picking of each other's feathers, especially under crowded conditions and if mineral deficiencies are present. Beak trimming at one-day-old chick is routinely used in layers and it is very effective in reducing plumage damage for long term as stated by Hartcher et al. (2015). On the other hand, the practice of beak trimming has recently been debated from an animal welfare perspective as it can cause pain and stress to the birds and future beak abnormalities

Figure 4.2 Open mouth of a male chicken (left), floor (middle), and roof (right) of the oropharyngeal cavity. The tongue (a) can be seen at the ventral aspect with the lingual papillae (b) at its base. Dorsally, the choana (d) communicates with the nasal cavity. The palatine papillae (c) separate the oral and pharyngeal cavities, not to be confused with the caudodorsal pharyngeal papillae (k) around the infundibular slit (j). Caudal to the caudal border of the laryngeal mound (e) and the glottis (f), a distinct row of conical papillae exists, the caudoventral pharyngeal papillae (g). The lateral palatine ridges (h) and the median swelling (i) can not be mistaken for papillae.

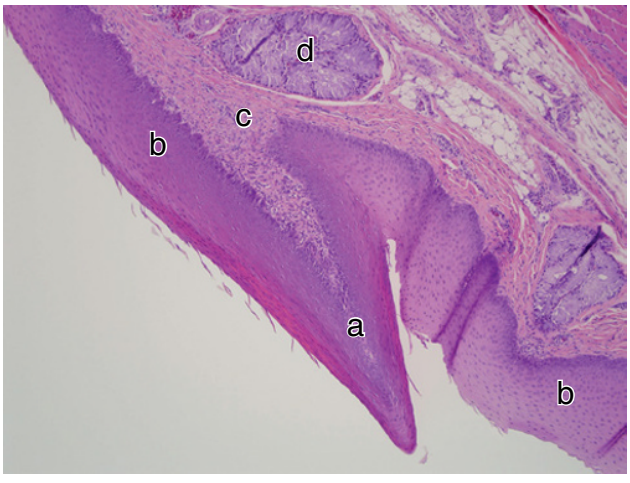

Figure 4.3 Palatine mucosa histological section. H&E stain. Palatine papilla (a) is oriented caudally, and they are covered with stratified squamous epithelium (b) with more keratin at the level of the papilla. Connective tissue (submucosa, c) and palatine salivary glands (d) are present underneath the epithelium.

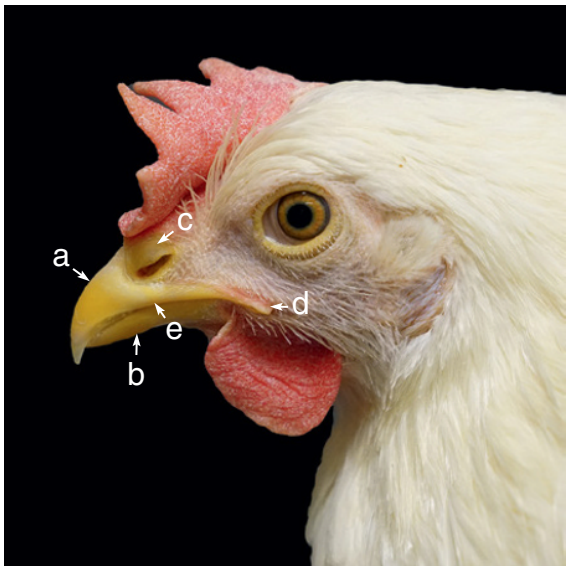

Figure 4.4 Left side view of the head of a female chicken. The beak is pointed at its tip and the upper and lower portions that meet at the angle of the mouth (d). The base of the beak is sharply convex in the upper portion where it forms the operculum (c) covering the external nares. The outer dorsal midline surface of the beak is the culmen (a) and the ventral midline ridge of the lower part of the bill (gnathotheca) is called gony (b). The cutting edges of the beak are called tomia (singular tomium) (e).

(Cheng 2006; Yamauchi et al. 2017). Other studies reported that minor beak trimming at a young age did not result in pain in young domestic chicks but led to impaired function of the magnetoreceptors and mechanoreceptors of the beak (Freire et al. 2011).

4.2.2 Tongue

The tongue (glossa, lingua) is a pointed organ in the chicken and has a triangular shape in a cross section, adapting to the space of the lower beak and jaw, where it lies. Its embryological origin is from the pharynx, where it has the root, although the greater portion of the tongue is located inside the oral cavity. The tongue is attached ventrally to the floor of the oral cavity by the lingual frenulum, a median fold of mucous membrane that does not reach the apex. When the beak is closed, the dorsal surface of the tongue (dorsum linguae) is applied to the palate, closing the rostral part of the choana and leaving only the caudal part open toward the laryngeal mound.

The tongue is covered by a stratified squamous epithelium on both surfaces, dorsal and ventral, with more organized and thicker keratinization on the ventral surface. The dorsal epithelial layer is relatively thick with many dermal papillae penetrating it. These dermal papillae result in islands of round structures of connective tissue with blood vessels and lymphatics because of incidence of sectioning (Figure 4.5). In the thinner ventral surface of the tongue, no dermal papillae are observed. The thickness of the stratified squamous epithelium anywhere in the animal body dictates the presence of these dermal papillae to facilitate nourishment of the thick avascular epithelial cells and supply them with oxygen. Underneath the epithelium, there is dense connective tissue surrounding the centrally located entoglossal cartilage in young birds and either fully or partially ossified in adult birds (Hodges 1974, p. 38). The entoglossal bone or cartilage is in continuation with the hyoid apparatus that can be found in the caudal lingual segment or root (hyolingual apparatus). Also, embedded in the connective tissue are salivary glands, present on both sides of the entoglossal cartilage or bone, as well as skeletal muscle.

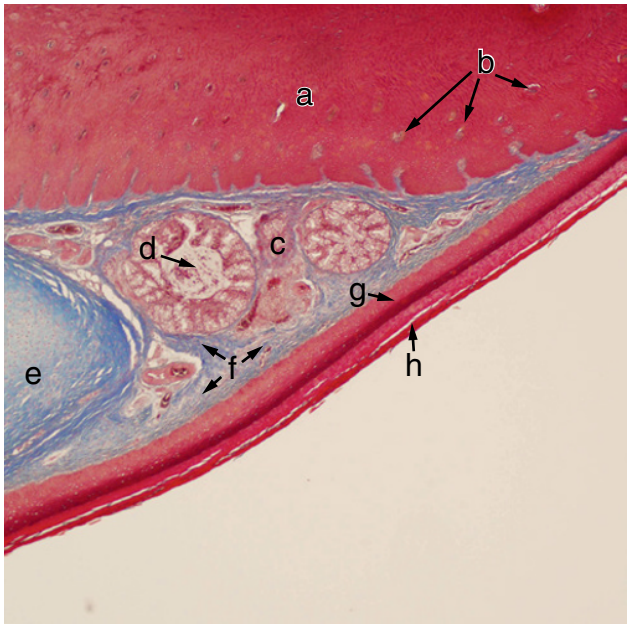

Figure 4.5 Histological transverse section of the tongue. Trichrome stain. The tongue is covered by a very thick stratified squamous epithelium on the dorsal aspect (a), with dermal papillae (b). The ventral surface (g) has more organized and thicker keratinization (h). Underneath the epithelium, a dense connective tissue (f) surrounding the centrally located entoglossal cartilage (e), has compound tubuloacinar lingual salivary glands (c) with a lumen (d).

4.2.3 Salivary Glands

The abundant and well-developed salivary glands of the chicken are compound tubuloacinar structures arranged continuously throughout all the walls (dorsal, lateral, and ventral) of the oral and pharyngeal cavities and located underneath the epithelium. These salivary glands drain through numerous openings that can be observed macroscopically (Figure 4.6). In the roof of the oropharyngeal cavity are the maxillary, palatine, and sphenopterygoid glands. In the lateral walls, the glands at the corner of the mouth and the cheeks are predominant, while in the floor of the mouth and pharyngeal cavities the mandibular, lingual, and cricoarytenoid glands are more abundant. In the chicken, the palatine glands are very abundant and are divided into medial and lateral groups (King and McLelland 1979, pp. 81–84; Samar et al. 2002) and the lingual glands are divided into rostral and caudal groups (Figure 4.6). All salivary gland consists of a variable number of lobules, each of which has several secretory tubules opening into a common cavity with a single duct that opens either directly into the lumen of the digestive system or into other ducts (King and McLelland 1979, pp. 81–84). The secretion inside the duct of the glands contains mucoid material with few glandular epithelial cells, fibroblasts, and relatively large numbers of plasma cells. The most common cell type in the secretory tubules of the chicken salivary glands is tall columnar after feeding or after 24 hours of fasting. These cells are filled with mucus, thus displacing the nucleus toward the basal pole of the cell. However, after a meal, time when the cellular content is released, the cells become narrow and small. The mucins inside the chicken salivary glands are sulfated and acidic mucopolysaccharides as in mammals. It has been described that chicken salivary glands don't secrete amylases, as it happens in other type of birds, because of the presence of a large crop where the food is stored for relatively long

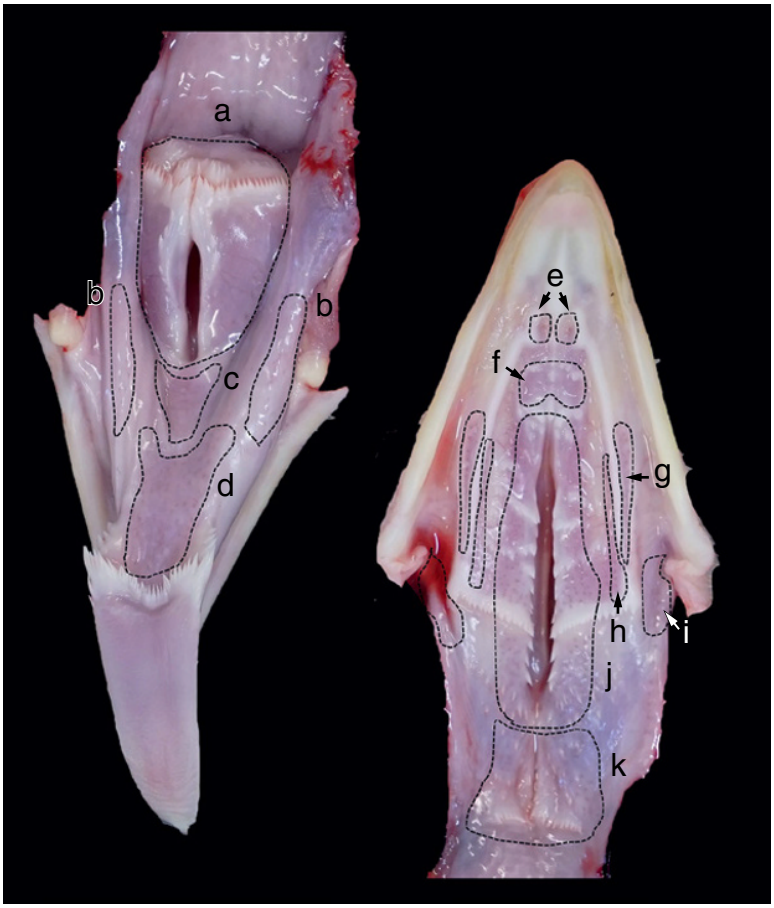

Figure 4.6 Floor (left) and roof (right) of the oropharyngeal cavity. In the floor of the mouth and pharyngeal cavities the glands are visible including the caudal mandibular (b), lingual (rostral (d) and caudal (c)) and cricoarytenoid (a) glands. In the roof of the mouth the openings of salivary glands (e), maxillary (f), lateral palatine (g), medial palatine (j), caudal mandibular of the upper jaw (h), and sphenopterygoid (k) salivary glands. The glands of the corner of the mouth and the cheeks are found in the lateral walls (i).

periods of time allowing ample opportunities for the plant amylases to act. The chicken salivary glands are involved in several functions. One is the lubrication and moistening of the food to protect the oral/digestive system surface and therefore facilitate passage of food through the long esophagus. It is also involved in hindering the proliferation of the buccal pathogenic flora acting as a non-immune protection mechanism (Samar et al. 2002).

4.2.4 Taste Buds

Chicken taste buds differ considerably from those of mammals in terms of shape and location. Chicken taste buds are ovoid-shaped, and they appear mostly associated to the openings of the salivary glands. Opposed to mammals, where taste buds are primarily located in the tongue, only 2% of chicken taste buds are in the posterior region of the tongue (Rajapaksha et al. 2016). The epithelium of the palate (70%) and the base of the oral cavity (30%, anterior mandibular gland region) are the main location of the taste buds in the chicken (Ganchrow and Ganchrow 1985; Kudo et al. 2008). The average number of taste buds varies depending on breed and gender but ranges from 240 to 360 (Rajapaksha et al. 2016).

4.2.5 Pharyngoesophageal Junction

Keratinization of the epithelium thickness and extent of glands decrease as the esophagus is approached. The thin layer of smooth muscle that comprises the lamina muscularis mucosae starts to appear at this junction. Lymphoid follicles or tonsils are also present in the pharyngoesophageal junction as described in the pharynx.

4.3 Hyoid Apparatus (Hyoglossal, Hyolingual, or Hyobranchial)

Because of the relationship with the tongue, the hyoid apparatus is discussed in this chapter. It is composed of several segments of bones (McLelland 1968) (Figure 4.7):

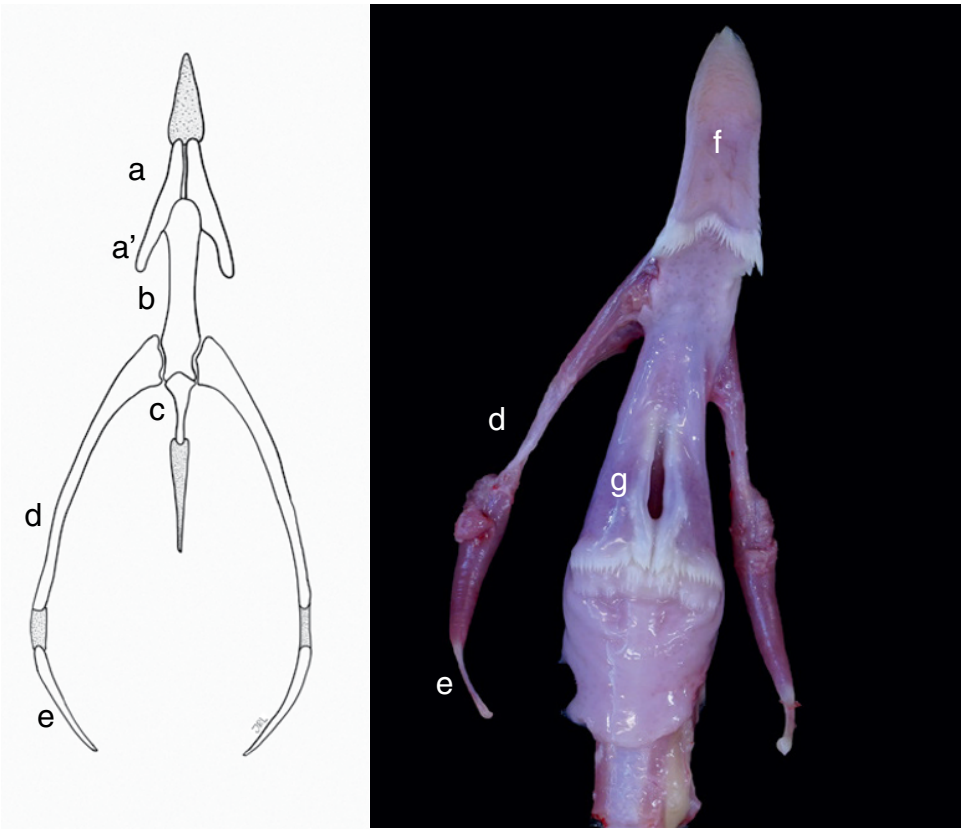

Figure 4.7 Dorsal view of the hyoid bone (left drawing based on McLelland 1968) and the tongue (f) and larynx (g) (photo right). Paraglossal (entoglossal) segment (a), which extends into the tongue (f) becoming hyaline cartilage. Paired lateral cornua (a') of the paraglossal bone (base of the tongue). Rostral (basihyal) (b) and caudal basibranchial (urohyal) bone (c). The ceratobranchial (d) and the epibranchial (e) bones form the rami of the hyoid. *Source:* Adapted from McLelland (1968).

1) Paraglossal (entoglossal) segment, which extends into the free portion of the tongue becoming hyaline cartilage.
   a) Paired lateral cornua of the paraglossal bone forming the wide base of the tongue.
2) Rostral basibranchial (basihyal) bone lies in the fixed portion of the tongue.
3) Caudal basibranchial (urohyal) bone.
4) Ceratobranchial bone.
5) Epibranchial bone.

4.4 Esophagus (Pars cervicalis, Crop, and Pars thoracica)

The esophagus is the muscular tube that connects the pharynx to the stomach. In the chicken, it has two distinct parts: the cervical and the thoracic parts separated by the crop (ingluvies). The cervical part connects the pharynx to the crop, while the thoracic portion connects the crop to the proventriculus. The chicken crop is relatively large in diameter and classified as a "real crop" compared with other avian species (Farner 1960; Proctor and Lynch 1993; Kierończyk et al. 2016). Researchers consider it an embryonic dilation of the esophagus before it enters the thoracic cavity.

The cervical esophagus is shorter than the length of the cervical vertebral column and, consequently, is displaced toward one side. Although the esophagus starts dorsal to the larynx and trachea, caudal to the fifth cervical vertebra, it lies on the right side of the neck. Close to the entrance into the thoracic cavity (cranial to the sternal rostrum and in front of the furcula, on the pectoral muscles), the esophagus returns to the midline and enlarges ventrally to form the crop. (Figure 4.8). In the *Gallus var. domesticus*, the ingluvies is characterized by thin wall, 4.5–5.0 cm of length and 8–10 cm^3 of capacity although it differs in shape and size among different breeds of poultry. The crop wall is attached to the skin and to the clavicle (clavicula, furcula) by loose connective tissue. It is also attached to the sternum by the musculus compressor ingluvialis (Kierończyk et al. 2016). The crop can store food for a short period of time. Since the crop is very superficial and, it is only covered by the skin, can be easily palpated when it is full

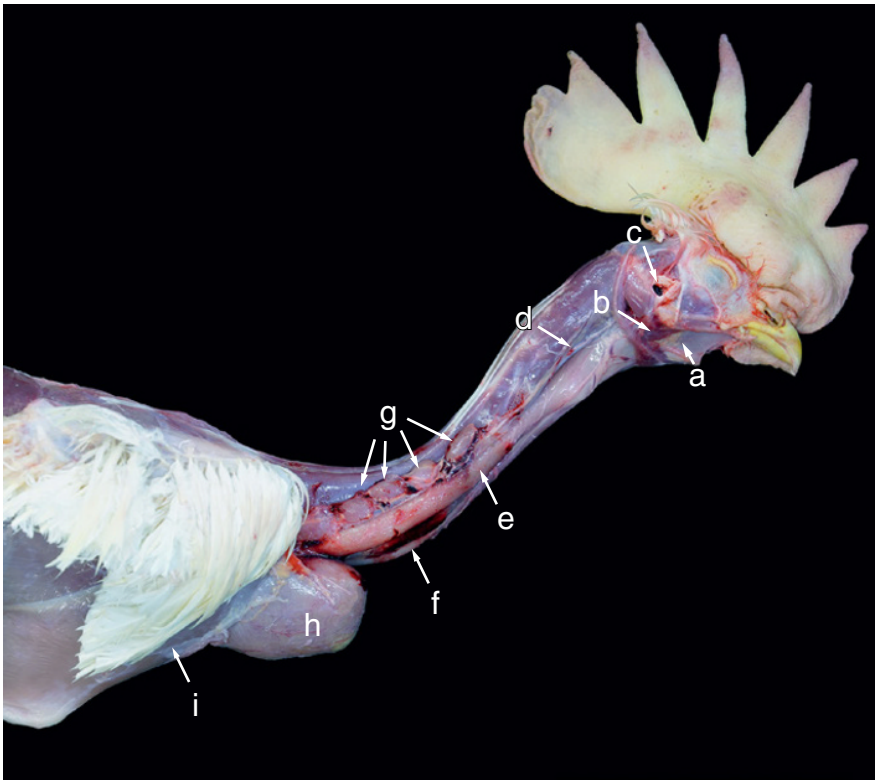

Figure 4.8 Right lateral view of the cervical and thoracic regions. (a) Pharynx, (b) depressor mandibularis muscle, (c) external acoustic meatus, (d) external jugular vein, (e) cervical esophagus, (f) trachea, (g) thymus, (h) crop, and (i) pectoral muscles.

(McLelland 1990). On the dorsal wall surface of the crop, there is a cleft, or a channel (crop channel) and easily digestible food may pass through it directly to the proventriculus like the gastric groove of the ruminants. The thoracic esophagus is much shorter than the cervical part and extends dorsal to the trachea along the base of the heart. The most caudal portion of the esophagus is reduced in diameter.

Histologically, the esophagus is lined by slightly keratinized stratified squamous epithelium and its wall has a folded appearance (Figure 4.9a). This esophageal epithelium appears very thick due to the large number of cell layers (Figure 4.9b). The esophagus has the typical layer arrangement of a tubular organ: tunica mucosa (lamina epithelialis, lamina propria, and lamina muscularis mucosae), tunica submucosa, tunica muscularis, tunica adventitia in the neck region, and tunica serosa in the thoracic region. The lamina propria is composed of loose connective tissue with large amounts of fibers that extend into the superficial epithelial layers (Figure 4.9c). This fact allows blood vessels to reach closer to the thick lining epithelium. Simple branched acinar glands which are mucous in nature are frequently observed in the lamina propria

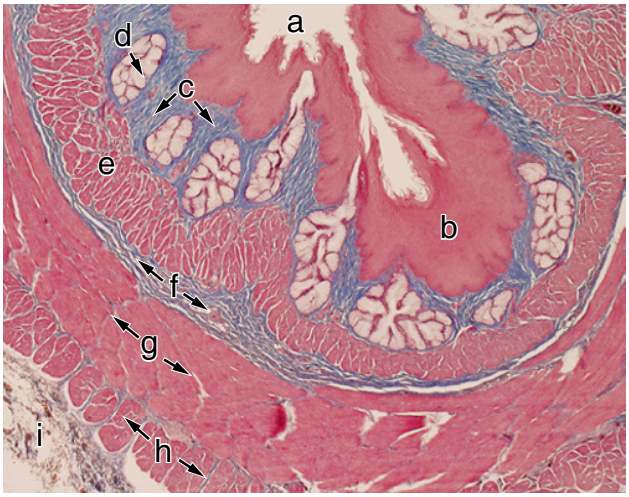

Figure 4.9 Section of the cervical portion of the esophagus. Trichrome. The lumen of the esophagus (a) is lined by stratified squamous epithelium (b) with the lamina propria (c) where mucosal glands are found (d). A thick lamina muscularis mucosae (e) separates the mucosa from a thin submucosa (tela submucosa) (f). The tunica muscularis is well developed with an inner circular layer (g) and an outer longitudinal layer (h). The tunica adventitia with blood vessels and nerves (i) is the most external layer.

(Figure 4.9d). The lamina muscularis mucosae is very well developed (Figure 4.9e) separating the glands from the submucosa. The lamina propria intermingles its connective tissue fibers with those of the tunica submucosa. The tunica submucosa is poorly developed and composed of loose connective tissue with blood vessels and nerves scattered throughout. The tunica muscularis is composed of an inner circular layer and an outer longitudinal layer. The direction of muscle fibers, especially within the inner layer, may differ from region to region and an oblique layer may appear in the wall of the esophagus. A thick layer of connective tissue infiltrated by large numbers of blood vessels and nerve fibers is the characteristic feature of the tunica adventitia in the cervical region (Figure 4.9i). The thoracic esophageal mucosa shares the characteristic features of the cervical portion except for the presence of a complete layer of lamina muscularis mucosae and few solitary lymph nodules present in the lamina propria. The outer tunica serosa is composed of a relatively thin layer of connective tissue covered by simple squamous epithelial cells (mesothelium).

The crop is lined by stratified squamous epithelium, which contains mucus glands at the crop channel only, and secrete the "crop milk." A cross section through the entire chicken crop wall reveals the following parts: incompletely keratinized stratified squamous epithelium, lamina propria, mucous glands (glandulae ingluviales), lamina muscularis mucosae, tunica submucosa, tunica muscularis with inner circular muscle and outer longitudinal layers, and tunica adventitia (McLelland 1990; Doneley 2010). Tunica mucosae ingluviei contains plicae and rugae ingluviei (McLelland 1993). The mucosa of the chicken crop is described to resemble the surface of the bovine rumen when examined with scanning electron microscopy (SEM) with a high ratio of surface per epithelial cell due to micropapillae (Bayer et al. 1975a, b). This fact in conjunction to the evidence that sugar rapidly decrease the crop while the amount of lactic acid, acetic acid, and ethanol increased (Bolton 1965; Pritchard 1972) which indicates the fermentative function of the crop with predominance of *Lactobacillus* population (Cousin et al. 2012).

4.5 Stomach (Proventriculus and Ventriculus)

The stomach of the chicken has two distinct parts separated by a constriction (isthmus) and consists of a cranial (oral) glandular stomach (proventriculus), smaller in size, and a caudal (aboral) muscular stomach (ventriculus, gizzard), larger in size.

4.5.1 Proventriculus (Glandular Stomach)

The proventriculus is connected to the esophagus cranially (orally) and to the muscular stomach (gizzard) caudally (aborally) (Figure 4.10). It is a fusiform structure of an average 4–5 cm in length and has no clear separation or

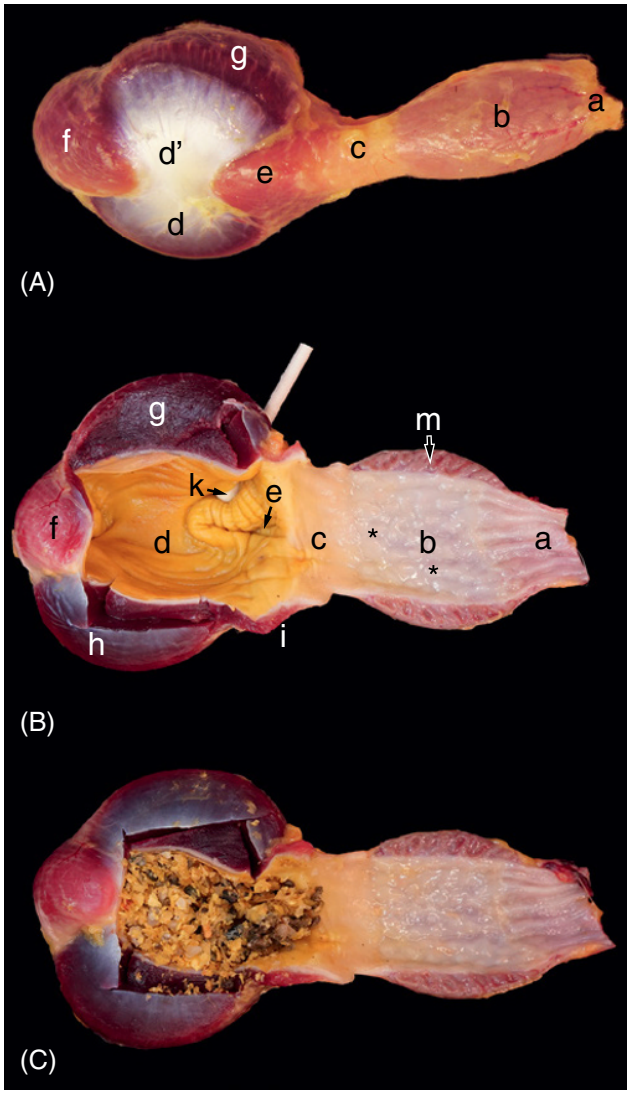

Figure 4.10 Chicken stomach seen from the left side. (A) External and (B and C) internal views (window on the right side of the gizzard). End of esophagus (a), proventriculus (b), intermediate zone (isthmus) (c), gizzard (d) with the tendinous center (d'), cranial blind sac (e), caudal blind sac (f), thick cranioventral muscle (g), thick caudodorsal muscle (h), and thin craniodorsal muscle (i). The pyloricoduodenal foramen (k) is seen close to the cranial blind sac. Large numbers of papillae are visible on the surface (asterisks) where the proventricular glands open. The thickness of the proventricular wall is occupied by these glands (m). (C) Open stomach with the presence of grit, small loose particles of stone or sand that help in the grinding of food, such as grain or vegetables, to become a digestible form.

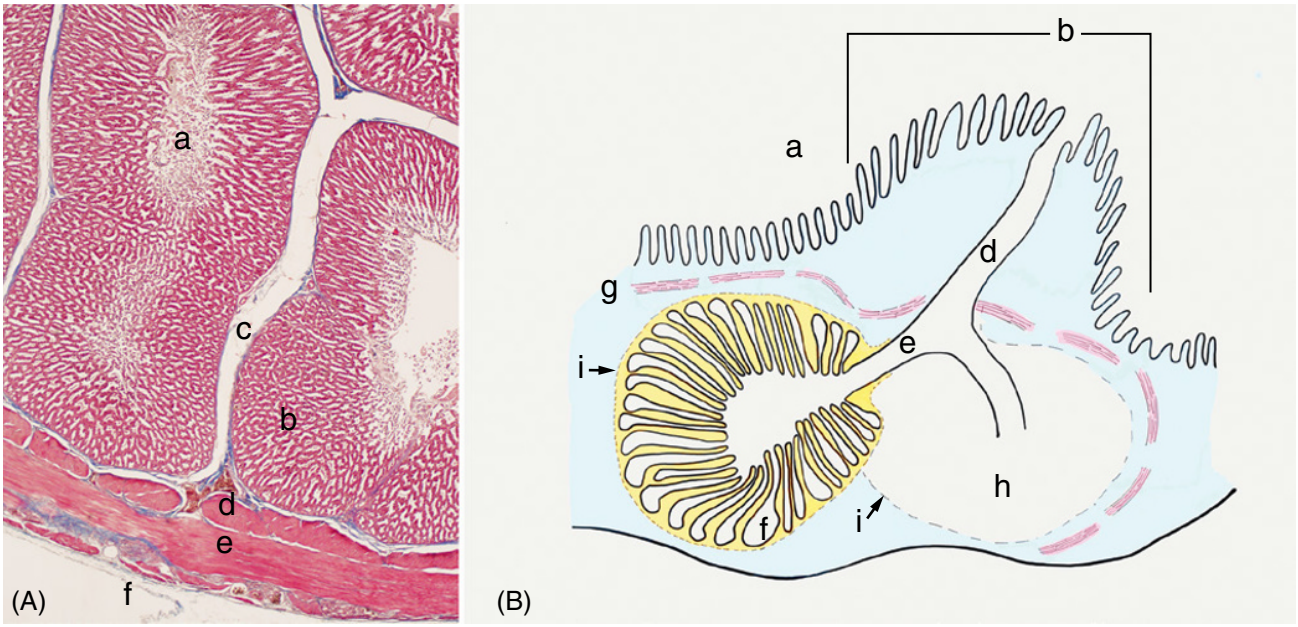

Figure 4.11 (A) Histological section of the proventriculus of the chicken stomach. Trichrome stain. Lumen (a), plicae and secondary ducts (b), separation of lobules with connective tissue septum (c), lamina muscularis mucosae (d), tunica muscularis (e), and tunica serosa (f). (B) Diagram of the chicken proventricular glands. *Source:* Redrawn from Hodges (1974). In the lumen of the proventriculus, (a) several raised papillae (b) are seen each of which has an opening (c) of a primary secretory duct (d). Each gland is composed of several glandular lobules (h) composed of several alveoli (f) that open into secondary ducts (e). The lobules are surrounded by a connective tissue septum (i) with collagen, elastic fibers, and few smooth muscle fibers. Lamina muscularis mucosae (g).

distinct sphincter at the esophageal junction. Located at the left lower quadrant of the body cavity, it is related to the left lobe of the liver and spleen. When the proventriculus is cut open, large numbers of papillae are visible on the surface as openings of the glands into the lumen (Figure 4.10).

The proventriculus has all the typical layers of a tubular organ (McLelland 1990). The epithelial lining is simple columnar and has many folds. The lamina propria is composed of loose connective tissue infiltrated with scattered lymphatic cells but, in circumscribed areas, these cells form well-defined lymphatic nodules which can extend into the tunica submucosa. Thin scattered layers of ill-defined lamina muscularis mucosae are present. Large compound tubular glands, the proventricular glands, almost completely occupy the tunica mucosa specifically at the level between the two layers of lamina muscularis mucosae (Aughey and Frye 2001; Eurell and Frappier 2006; Bacha and Wood 2012) (Figure 4.11A).

The mass of proventricular glands constitutes most of the thickness of the proventricular wall. The glands are composed of numerous glandular lobules surrounded by a connective tissue septum with collagen, elastic fibers, and a few smooth muscle fibers (Calhoun 1954) (Figure 4.11B). Each lobule is constituted of several alveoli arranged peripherally that open into secondary ducts, several of which will open into a primary secretory duct draining into the lumen of the proventriculus through one of the mucosal papillae (Figure 4.11B). The epithelial lining of these glands is simple cuboidal with a single cellular type detected by routine stain (H&E). These cells are called oxyntic peptic cells, which secrete both pepsin and hydrochloric acid. These glands have excretory ducts, which are filled with fluid, glandular epithelial cells, a few blood cells, and cellular debris.

The tunica submucosa is very much reduced and composed of bundles of connective tissue, which are infiltrated by blood vessels, and extends between the glands (Figure 4.11A). The inner layer of the muscular tunic appears to be part of the displaced layer of the lamina muscularis mucosae. Therefore, the actual tunica muscularis is composed of a well-developed thick inner circular layer and an incomplete outer longitudinal layer. Myenteric plexus and ganglia, as well as blood vessels, are clearly seen between these two layers or within the outer layer. Small, scattered lymphoid follicles or aggregations are also present in this region. The outer thin connective tissue elements are covered by mesothelium (simple squamous epithelium).

4.5.2 Intermediate Zone

The intermediate zone, which is also called the isthmus, is the area of connection between the proventriculus and the gizzard (Figure 4.10). This segment is relatively short and has

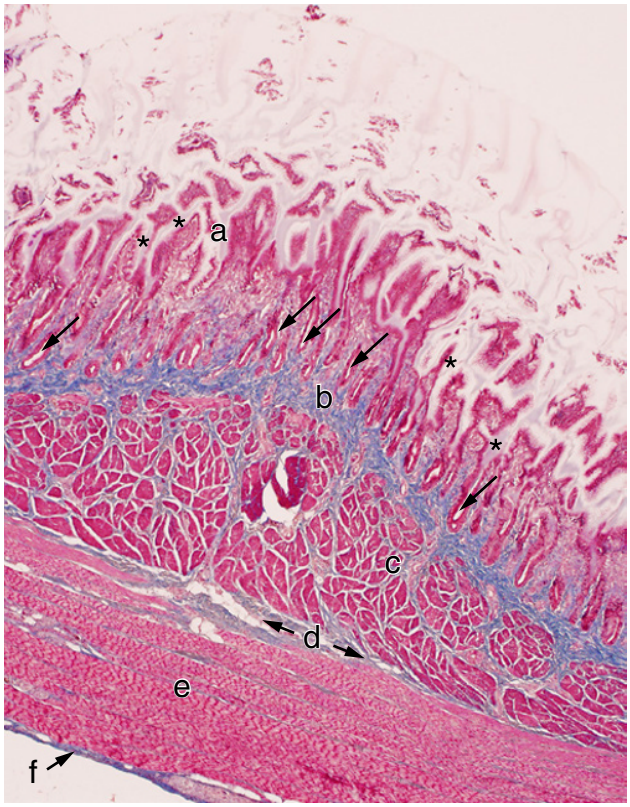

Figure 4.12 Histological section of the intermediate zone (isthmus) of the chicken stomach. Trichrome. The epithelium is simple columnar (a) with tubular glands (arrows) producing a mucoid secretion that mixes gizzard glandular secretion and creating a "horny layer" (asterisks). The lamina propria (b) separates the epithelium from the extensive lamina muscularis mucosae (c). A small tunica submucosa (d) separates the tunica muscularis (e). The outer most layer is the tunica serosa (f).

the characteristic features of the wall of the gizzard with some variations. There is a sudden decrease of proventricular mucous glands and, the secretions observed in this segment is a mix mucoid secretion and gizzard glandular secretion. The tunica muscularis consists of a thin outer layer of longitudinal muscle and a thick middle layer of circular muscle. In addition, a layer of lamina muscularis mucosa, of about half of the thickness of the circular layer, is also present separated by a scattered and thin tunica submucosa (Figure 4.12). The outer longitudinal layer thins up and disappears in the transition to the gizzard while the inner circular and the lamina muscularis mucosae fuse together to form the main mass of the gizzard muscle (Hodges 1974, pp. 53–63).

4.5.3 Gizzard (Muscular Stomach, Ventriculus)

The gizzard is a muscular flattened structure that connects the proventriculus (cranially) to the duodenum (ventral and to the right of the gizzard). It is the largest portion of the chicken stomach and, as in all birds, acts as the "masticatory organ" due to lack of teeth (Moore 1999; Hetland Svihus and Krogdahl 2003). It is located at the ventral left quadrant of the peritoneal cavity and it is related ventrally with the liver (mainly left lobe), cranially with the spleen, caudomedially with different intestinal segments, and in females dorsally with the oviduct (Figure 4.14).

The shape of the gizzard is quite irregular (biconvex lens) with the greater diameter align to the longitudinal body axis, resulting in a right and left surfaces with a distinct central aponeurotic region (tendinous center). The central part of the gizzard is the body (corpus) that separates the cranial and caudal sacs.

The gizzard is composed of four muscular units that can be externally differentiated but all of them are connected to the central aponeurotic regions (tendinous centers) (Figure 4.10). These four muscular compartments are named cranial dorsal and ventral and caudal dorsal and ventral and are also referred to as thin or thick depending on their size. Hence, we can name them as thin craniodorsal, thick cranioventral, thick caudodorsal and thin caudoventral muscles (King and McLelland 1979, pp. 115–123) (Figure 4.10). Internally, the surface of the gizzard is covered by a thick keratinized yellowish layer (koilin, cuticle), which can easily be stripped from the surface, as it is made of hardened glandular secretions (Figure 4.10B). The yellowish coloration is due to the antiperistaltic movements occurring at the small intestine, which bring contents from the beginning portion of the duodenum that has a high concentration of bile pigments. The koilin creates some ridges that, with the addition of small, adhered grits or stones, help with the grinding of the ingested grain.

The epithelium lining the mucosa is simple low columnar, highly keratinized, which some authors call the "horny layer of the stomach." This keratin extends deep inside the pits on the gizzard surface epithelium. The lamina propria has branched tubular glands (Figure 4.13) that differ in their distribution from region to region. Two types of cells can be detected with routine stain in these glands: one type predominates within the glands, while the other type appears as a small group of lightly stained cells at the base of the glands. The lamina muscularis mucosae is displayed as scattered bundles of smooth muscle cells arranged longitudinally. The glands of the lamina propria push these lamina muscularis toward the tunica muscularis as seen in the proventriculus. The tunica submucosa is represented by an extremely thin layer of connective tissue that separates the lamina muscularis mucosae from the inner layer of the tunica muscularis. The thickness of the tunica muscularis varies from region to region in the wall of the gizzard. In general, it is composed of a thick inner circular layer and a thick outer layer of longitudinally arranged

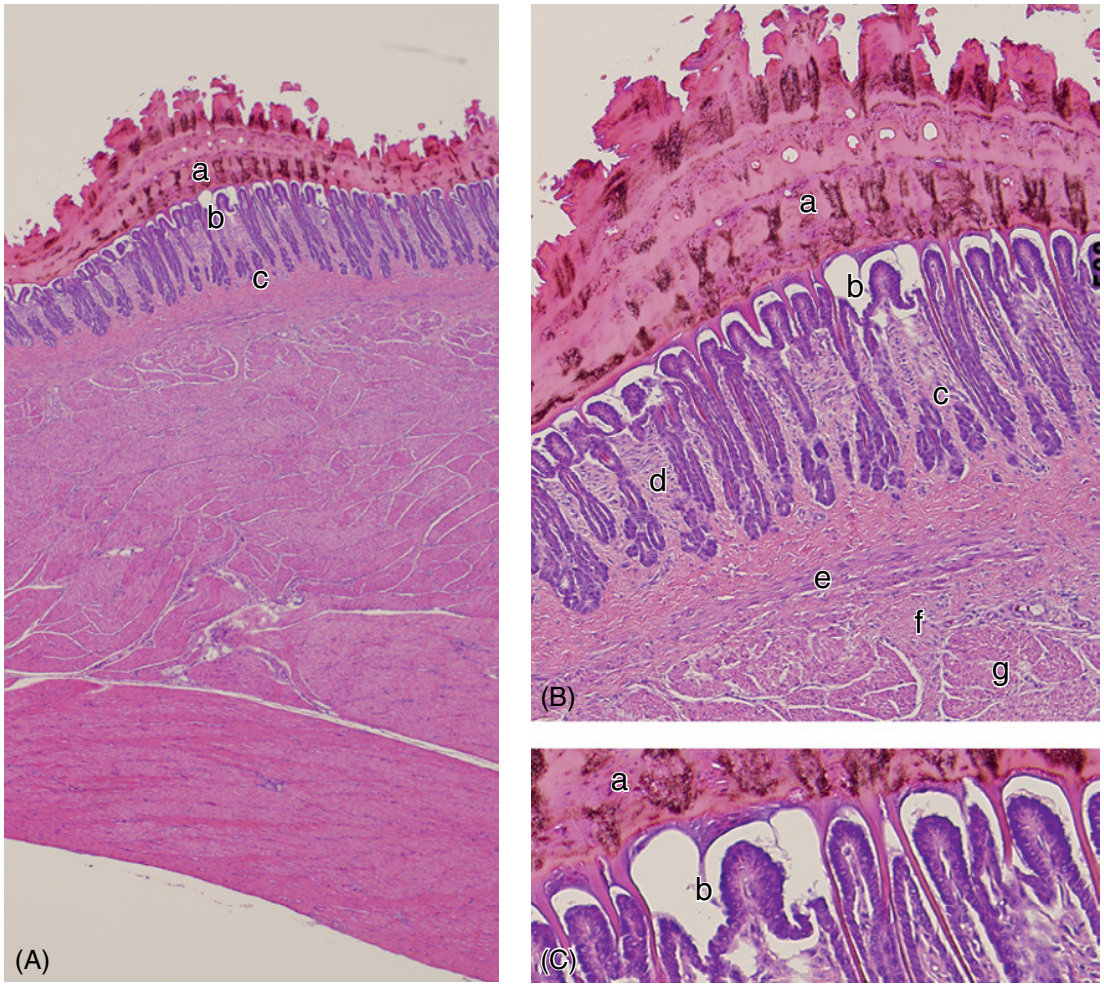

Figure 4.13 Gizzard (ventriculus). H&E stain A, B, and C. Koilin (a), surface epithelium (b), glands within the lamina propria (c), lamina propria (d), lamina muscularis mucosa (e), tunica submucosa (tela submucosa) (f), and Internal muscular layer (g).

bundles of smooth muscle cells. These bundles are separated or covered with a layer of connective tissue, which is infiltrated by blood vessels and nerve fibers (Figure 4.13). The outermost layer, which is the tunica serosa, is composed of connective tissue, adipose tissue, blood vessels, nerve fibers, and lymphatics. It is covered from outside by simple squamous epithelium. Terminal ganglia (parasympathetic) are seen in this layer along the course of the nerve bundles. The innervation of the gizzard can be complex (Gabella and Halasy 1987). The musculature receives vagal (cholinergic) innervation, while a sympathetic (adrenergic) innervation is well developed around intramuscular blood vessels.

Most chicken that are raised cage free or roam in pastures ingest grit, small loose particles of stone or sand, that are housed inside the gizzard (Figure 4.10C). Many of the grit stones eaten pass through the small intestine without being retained in the gizzard (Svihus et al. 2017). They help in the grinding of food, such as grain or vegetables, to become a digestible form. The shape of grit in the gizzard depends on the diet and gizzard muscularity of chicks (Takasaki and Kobayashi 2020). The shape and quantity of the grit in the gizzard is greatly modified through abrasion indicating that grit does not retain its original sizes nor shapes upon ingestion. The composition of the grit stones is important for their function (Svihus et al. 2017).

4.6 Small Intestine

The small intestine consists of three segments, duodenum, jejunum, and ileum (Figure 4.14). These three regions cannot be clearly differentiated grossly or histologically but it is agreed that the jejunum starts after the distal limb of the duodenum when crosses over the mesenteric artery and based on the blood supply, the beginning of the ileum is marked by the apices of the two ceca. As in the case of mammals, the epithelial of lining the chicken intestine

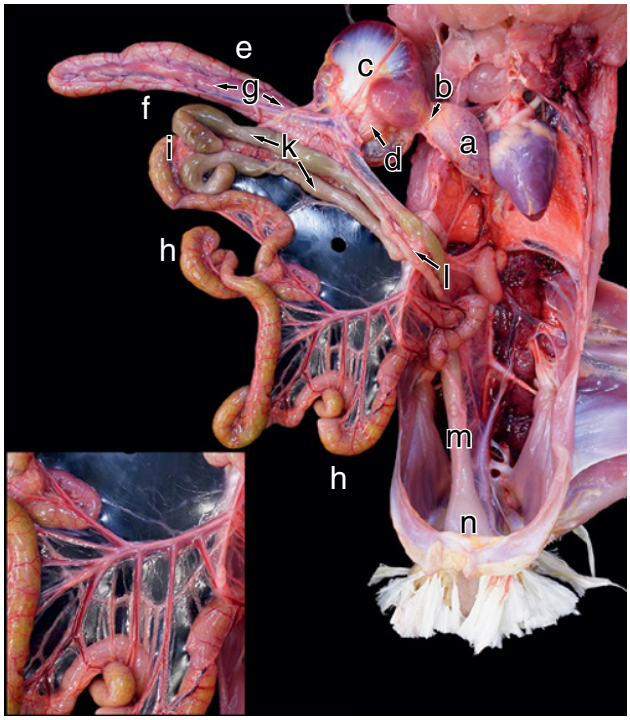

Figure 4.14 Abdominal organs of a male adult chicken (liver and spleen are removed). (a) Proventriculus, (b) isthmus, (c) gizzard, (d) beginning of duodenum at the pyloricoduodenal junction, (e) descending duodenum, (f) ascending duodenum, (g) pancreas, (h) jejunum, (i) beginning of ileum, (k) ceca, (l) ileocecal junction, (m) rectum, and (n) cloaca. Inset, blood supply to the small intestine.

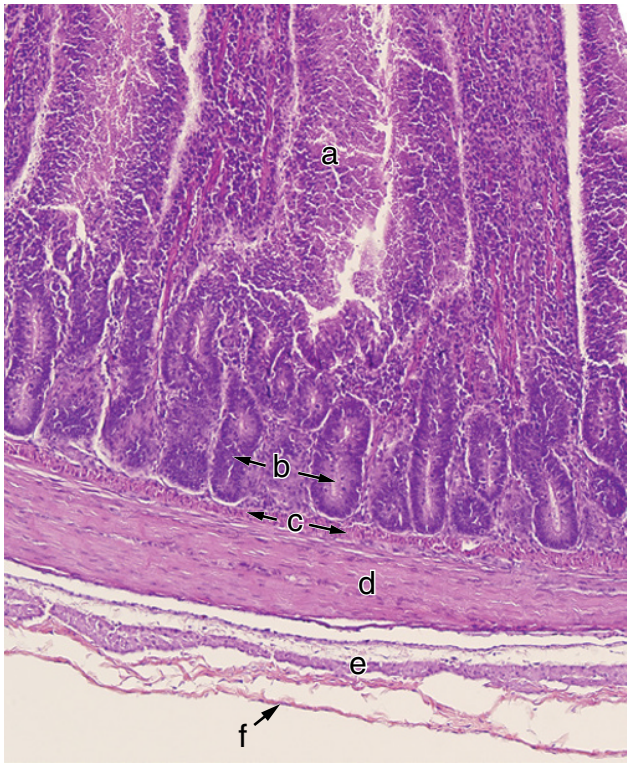

Figure 4.15 Histological section of the chicken duodenum. H&E stain. Villi (a) are covered with a simple columnar epithelium with goblet cells. The lamina propria is packed with simple branched tubular glands (b). There is a well-developed longitudinally arranged lamina muscularis mucosae (c), which prevents further extent of the glands toward the submucosa and contract to help release the secretion from the glands. The tunica submucosa is very much reduced and appears to be absent in some areas because the lamina muscularis mucosae is in direct contact with the tunica muscularis inner layer (d). The outer longitudinal layer of the tunica muscularis is reduced (e). The tunica serosa is composed of bundles of connective tissue fibers (f).

have villi and intestinal crypts. Epithelial cells of the villi have about 10^5 microvilli per square millimeter on their apical surface to increase the absorbing surface area by 15-fold (Klasing 1999).

4.6.1 Duodenum

The duodenum is the first segment of the small intestine and starts at the gizzard (at the pyloroduodenal ostium) and ends at the jejunum. It forms a long, well-defined loop with descending (proximal) and ascending (distal) portions (limbs). These two portions are separated by the duodenal flexure, and they are connected by a fold of peritoneum (interduodenal ligament). The pancreas is lodged between these two portions and extends the entire length of the duodenal loop (Figure 4.14). The site of termination of the ascending duodenum is at the beginning of the jejunum and it is where the duodenal papilla can be seen protruding on the mucosal surface at the opposite side of the gizzard, with the openings of the pancreatic and bile ducts.

The surface of the duodenal mucosa is characterized by the presence of villi that develops rapidly from day 1 after hatching (Bayer et al. 1975a, b; Sklan 2001; Uni et al. 2003). The length and frequency of these villi vary depending on the age and breed of chickens (Yamauchi and Isshiki 1991). The epithelial lining of the duodenal villi is simple columnar with goblet cells. The lamina propria is packed with simple branched tubular glands. There is a well-developed longitudinally arranged lamina muscularis mucosae, which prevents further extent of the glands toward the submucosa. The tunica submucosa is very much reduced and appears to be absent in some areas because the lamina muscularis mucosae is in direct contact with the tunica muscularis inner layer. The tunica serosa is composed of bundles of connective tissue infiltrated by large numbers of blood vessels and nerve fibers. It is covered by simple squamous epithelium (Figure 4.15). The myenteric plexus and ganglia are observed within the outer layer of the tunica muscularis. Few scattered neurons and nerve fibers are found in the tunica submucosa representing the submucosal plexus and ganglia.

4.6.2 Jejunum

The jejunum is the second part of the small intestine which lies between the duodenum and the ileum. Within the abdomen, it begins opposite the muscular stomach where the bile and pancreatic ducts open into the duodenum. The mesentery of the jejunum is extensive (Figure 4.14). Meckel's diverticulum (or vitelline diverticulum) is a short blind remnant of the yolk sac and yolk stalk which has been traditionally used to indicate the line of demarcation between the jejunum and the ileum. However, the long mesentery of the jejunum and the intestinal vascular pattern are more compelling characteristics to differentiate these two segments of the small intestine. Meckel's diverticulum has a myelopoietic function in adults. So, it will be discussed in Chapter 11 (The lymphatic system) with the lymphoepithelial organs. The transition of the jejunum to the ileum is difficult since the external diameter barely changes.

The intestinal villi present in the intestinal mucosa of chickens gradually decrease in length (Rolls et al. 1978) and size (Altmann and Leblond 1970) moving from the duodenum to the ileum (Yamauchi et al. 2010). The epithelial lining of the jejunum is simple columnar with goblet cells and the surface is highly folded. Simple tubular glands fill the lamina propria. Microvilli are clear on the surface with the line of desmosomal attachments evident with high power resolution. Small bundles of smooth muscle cells project into the villi of the jejunum. Blood vessels fill the lamina propria and extend into the villi reaching close to the surface epithelium. Relatively thick and well-circumscribed lamina muscularis mucosae is present. However, very ill defined tunica mucosa appears only where blood vessels or nerve fibers infiltrate. A thick inner circular layer of the tunica muscularis and a thin outer longitudinal layer are evident (Figure 4.16).

Medium to relatively large muscular blood vessels with nerve fibers occupy the area within the outer layer and between the outer and inner layers of the tunica muscularis. Tunica serosa is represented by a well-defined zone of connective tissue covered from outside by mesothelium (simple squamous epithelium).

4.6.3 Ileum

The ileum is relatively short, and it is the last segment of the small intestine. It is lodged between the two ceca to which it is attached by a fold of peritoneum called the ileocecal ligament or fold. The ileum has a relatively thicker wall when compared to the duodenum and jejunum due to the extensive muscular layer and villi that reduces the lumen. The epithelial lining is simple columnar with large numbers of goblet cells and less enterochromaffin cells.

Figure 4.16 Histological section of the jejunum. Trichrome stain. Villi (a), simple branched tubular glands (b), lamina muscularis mucosae (c), tunica muscularis inner layer (d), outer longitudinal layer of the tunica muscularis (e), and tunica serosa (f).

The microvilli are well developed and appear in few instances as cilia in cross section. Simple branched tubular glands that fill the lamina propria are evident in the ileum. A few lymphoid aggregations are observed in the lamina propria, though they do not form organized nodules. The lamina muscularis mucosae is well developed and composed of a single layer of longitudinally arranged muscle fibers (Figure 4.17). The tunica submucosa is very much reduced, and it appears clearly only in areas where large blood vessels enter or leave this layer. A few scattered ganglionic neurons among bundles of axons are present in this layer (submucosal plexus and ganglia). The tunica muscularis is composed of a thick inner circular muscular layer and a relatively thin outer longitudinal muscular layer. In between the two layers, the myenteric plexus and ganglia are present. The tunica serosa is covered with simple squamous epithelium beneath loose connective tissue filled with blood vessels and nerve fibers. These blood vessels, as well as others supplying the wall of the ileum, contain large numbers of lymphocytes in addition to other blood cell types. At the mesenteric attachment, there are large quantities of adipose tissue in the tunica serosa. This area is the site of entrance of the blood vessels and nerves into the organ.

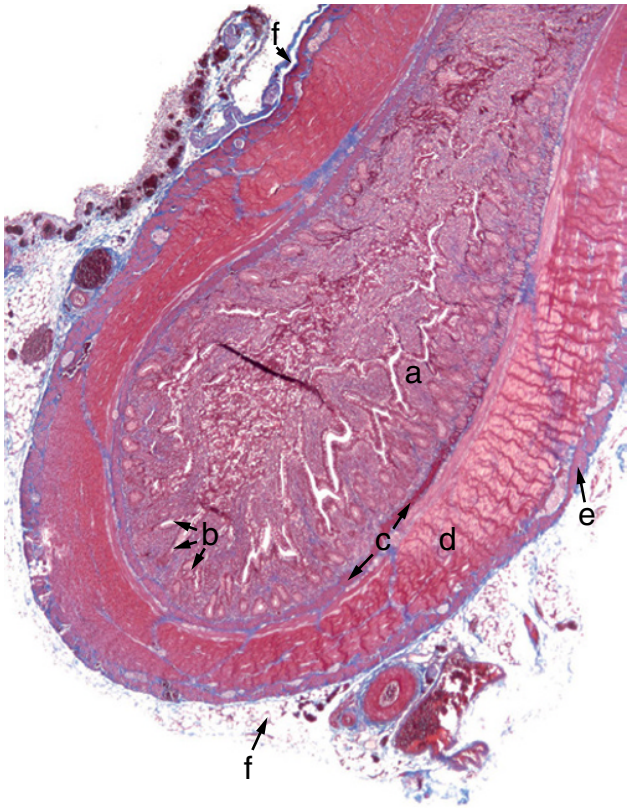

Figure 4.17 Histological section of the ileum. Trichrome stain. Villi (a), simple branched tubular glands (b), lamina muscularis mucosae (c), tunica muscularis inner layer (d), outer longitudinal layer of the tunica muscularis (e), tunica serosa (f).

4.7 Large Intestine

4.7.1 Rectum (Colon, Colorectum)

Following the Nomina Anatomica Avium (Baumel et al. 1993), this segment of the large intestine from the ileocecal junction to the cloaca will be referred to as rectum, but formerly was known as colon or colorectum (Figure 4.14). Compared to mammals, it is a short tubular structure in the chicken (around 8-cm long), and it is straight in its course.

The chicken rectal mucosa is folded, with abundant villi and lined by simple columnar epithelium with large numbers of goblet cells. Bundles of smooth muscle fibers extend inside the villi, originating from a well-developed lamina muscularis mucosae. A line of microvilli appears on the surface of these villi throughout most of the colon. The lamina propria is composed of loose connective tissue and is highly populated by cells of connective tissue origin including fibroblasts, lymphocytes, and plasma cells. In addition, simple branched tubular glands are also seen (Figure 4.18). Tunica submucosa is also present, but it is thin and filled with blood vessels, nerve fibers, and ganglia. The colon has a thick tunica muscularis with an inner circular layer that is much thicker than the outer longitudinal layer. The outer layer, though, is well arranged and better organized than the one in the wall of the cecum. Myenteric plexus, ganglia, and blood vessels are abundant between

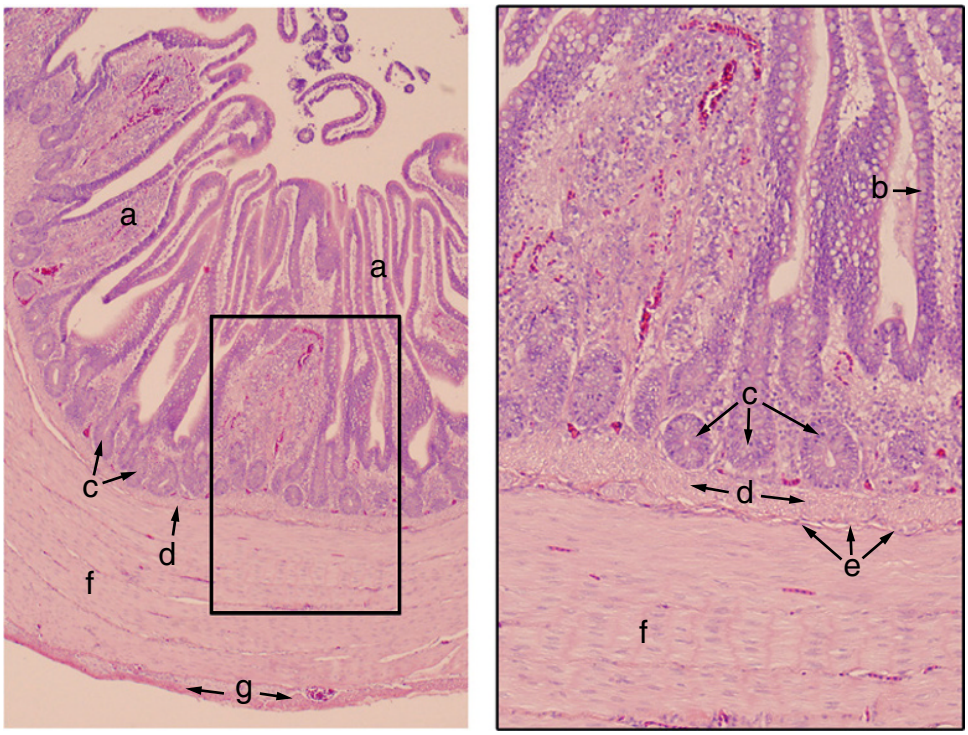

Figure 4.18 Histological section of the rectum (colorectum). H&E stain. The photo of the right corresponds to the inset. Villi (a), simple columnar epithelium with goblet cells (b), simple tubular glands (c), lamina muscularis mucosae (d), scarce tunica submucosa (e), tunica muscularis with an inner circular layer (f), and a small outer longitudinal layer (g).

the two layers of the muscular tunic. The tunica serosa is a relatively thin layer composed of connective tissue covered by simple squamous epithelium.

4.7.2 Ceca

The chicken ceca are a pair of blind end sacs (tubular sacs or ducts), directed backward along the ileum, and attached to this segment by a short fold (ileocecal fold) (Figures 4.14 and 4.19A). Their main function is to digest particles through the microflora as well as for the conservation of water and/or nitrogen recycling (DeGolier et al. 1999; Karasawa 1989). The openings of the two ceca are located at the junction of the ileum with the rectum. The ceca are smaller in diameter at their origin and gradually become wider as they ascend into the middle and blind end or apex. In 6-week-old chickens, each cecum is approximately 9-cm long (Ferrer et al. 1991).

The wall is thick at the origin and much thinner toward the blind dilated ends. Located 2–3 mm from the cecal origin, there are the cecal tonsils (del Cacho et al. 1993), a lymphoid organ that will be discussed in Chapter 11 (The lymphatic system).

The surface of the cecal mucosa is formed by spatulated villi of variable heights with a conical shape (Ferrer et al. 1991). The ceca are lined by highly folded simple columnar epithelium with relatively large numbers of goblet cells. Simple branched tubular glands extend down into the lamina propria, which is composed of loose connective tissue. An interrupted (incomplete) thin layer of lamina muscularis mucosae is present and it is separated with the tunica muscularis by a poorly developed tunica submucosa. Hence, tunica propria submucosa is the proper term when there is an absence of the lamina muscularis mucosae. Thus, it is difficult to differentiate the transition of connective tissue from lamina propria to tunica submucosa. The tunica muscularis is relatively thin at the dilated ends (apex) of the ceca and thicker at the level close to the ileocecocolic junction. As in the case of the other segments of the intestine, the tunica muscularis is composed of an extremely thick inner circular layer and a very thin outer longitudinal layer. Blood vessels, lymphatic vessels and nerve fibers are present in between these two layers, as well as in the submucosa and the lamina propria.

4.8 Cloaca

The cloaca is the terminal dilated chamber where the digestive and urogenital systems empty. Cranially connects to the rectum and caudally opens into the vent (Figure 4.20). The cloaca has three distinct compartments separated by mucosal folds. The first, most cranial, compartment is connected to the rectum and is called the coprodeum. The second compartment is the urodeum, which receives the two ureters and the genital system (oviduct in the hen or ductus deferens in the rooster). The third compartment is the proctodeum, the most external of the three compartments that connects to the vent. Grossly, the coprodeum is not clearly separated from the rectum (no well-developed fold of mucosal membrane separating); therefore, some authors consider the division based on the embryonic development or considering the cloacal shape or diameter

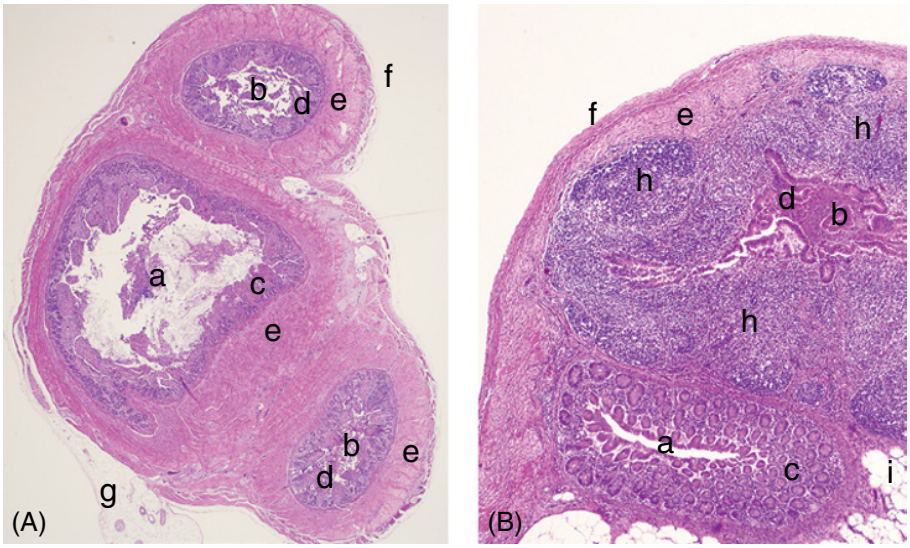

Figure 4.19 (A) Histological section of the ileum (a) and ceca (b) H&E stain. (B) Higher magnification of the cecal wall at the level of the cecal tonsil (h). The mucosa of both segments is lined by a simple columnar epithelium with goblet cells (c, d). The tunica muscularis with an inner circular layer (e) and a small outer longitudinal layer (f). Accumulations of adipocytes associated with the mesentery of the ileum (i).

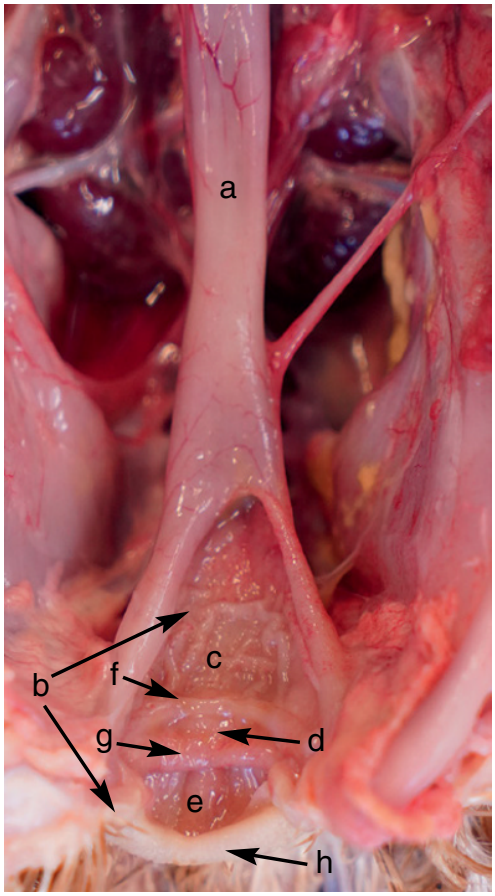

Figure 4.20 Ventral view of the chicken final portion of the digestive tract. The rectum (a) dilates into the cloaca (b). The most cranial division is the coprodeum (c) followed by the urodeum (d) and the most caudal is the proctodeum (e). Although it is incomplete, a circular coprourodeal fold (f) separates the coprodeum from the urodeum, just as the uroproctodeal fold (g) separates the urodeum from proctodeum. Dorsal lip of the vent (h).

in the adult chicken. Although it is incomplete, a circular coprourodeal fold separates the coprodeum from the urodeum, just as the uroproctodeal fold separates the urodeum from proctodeum.

The inner surface of the cloaca has folds lined by simple columnar epithelium with goblet cells (Figure 4.21). A small number of simple straight tubular glands open into the surface epithelium and extend into the underlying lamina propria. In addition to the normal cells present in the typical epithelium of the glands, Paneth cells, which have a rounded nucleus, are found at the base of the gland. These cells contain granules that stain pink to red in color with hematoxylin and eosin stain. The lamina propria is highly vascular and heavily infiltrated by cells of connective tissue origin. There is a well-developed lamina muscularis mucosae, which follows the folds of the cloacal wall and, occasionally, penetrates deep into the folds. This lamina muscularis mucosae is clearly divided into inner circular and outer longitudinal layers. The tunica submucosa is a clearly defined layer with loose connective tissue, blood vessels, nerve fibers, and ganglia. Thick irregularly arranged tunica muscularis is also observed. However, in areas of low folds, this layer is divided into a thick inner circular and a thinner outer longitudinal layer. In between these layers, blood vessels, nerve fibers, and ganglia are abundant. The tunica serosa covers most of the cloaca with the exception of the most distal (caudal, aboral) part, which is connected to the vent that is covered by connective tissue adventitia. Large muscular size arteries, veins, nerve bundles, and ganglia are abundant in this tunic. They are usually present at the mesenteric border of the cloaca. The epithelium of the terminal portion of the cloaca becomes stratified squamous epithelium as it opens into the vent. At the junction with the vent, the wall of the cloaca is still

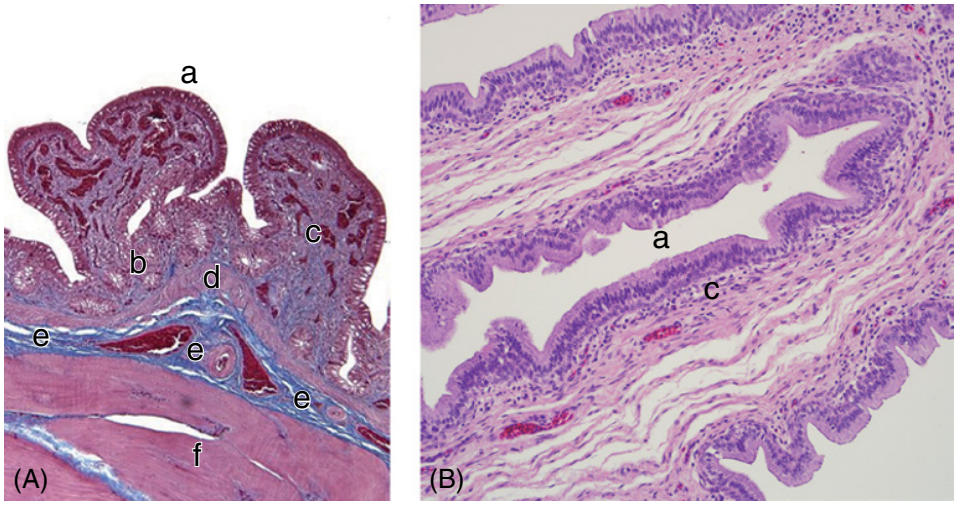

Figure 4.21 Histological sections of the female cloaca. Trichrome stain (A). H&E stain (B). The cloaca is lined by simple columnar epithelium with goblet cells (a). A small number of simple straight tubular glands (b) can be seen in the lamina propria (c) that is highly vascular. A well-developed lamina muscularis mucosae (d) separates the tunica submucosa (e) with abundant blood vessels, nerve fibers, and ganglia. Thick irregularly arranged tunica muscularis is also observed (f).

lined by simple columnar epithelium and the propria submucosa contains simple branched tubuloacinar glands. The lamina propria-submucosa is highly infiltrated by lymphocytes and well-circumscribed nodules appear close to the surface epithelium. The lamina muscularis mucosae disappears in this region. The lamina propria-submucosa is composed of loose connective tissue, highly vascularized and contains little adipose tissue in its deeper layer close to the tunica muscularis. The tunica muscularis becomes skeletal muscle instead of smooth muscle at this junction. It is composed of thick, regularly arranged fibers or bundles separated by connective tissue.

4.9 Vent

The external opening of the digestive system, as well as the genitourinary, is the vent. The vent has a dorsal and a ventral lip (labii) and a labial cleft between the two (Figure 4.22A). An abrupt change in the type of epithelium is observed at the junction of the distal portion of the cloaca and the vent. The epithelium is stratified squamous epithelium at the vent (Figure 4.22B). The cells at the surface are lightly stained and a little larger than other cells on the inner surface of the vent. As they move to the outer surface of the vent, these cells become flat and start to lose nuclei and a little keratin appears on the surface epithelium. The keratin thickness tends to increase toward the lip of the vent.

The dermis consists of dense irregular connective tissue; however, regularly arranged collagen bundles are observed in parallel alignment to the epithelial surface. The dermis is highly vascularized and contains large numbers of blood vessels, mainly arterioles and capillaries. Few arteriovenous anastomoses are observed in the dermis. At this level, Herbst corpuscles are abundant close to the surface epithelium (King 1975; Bacha and Wood 2012). Skeletal muscle bundles are clearly seen and well developed around the vent region. Most of these muscle fibers run longitudinally although a few circular and oblique bundles are also observed. The thickness of the epithelium as well as the keratin layer increases toward the skin.

4.10 Liver (Hepar, Jecur)

The liver in the adult chicken may appear reddish, light brown, or yellowish depending on the nutritional status, general health, and the method of sacrifice (Nickel et al. 1977). The color of the liver in young chickens, just after hatching, is yellowish as it has pigments carried with the lipids from the yolk. It is a big organ in chicken and in normal conditions weights between 30 and 50 g and represents 1.7% to 2.3% of the total body weight (King and McLelland 1979, p. 156). The liver can be seen when the abdominal wall is cut open and is divided into two lobes (left and right) with the right lobe being larger than the left. The left lobe is clearly divided into medial and lateral portions (Lucas and Denington 1956; Nickel et al. 1977, p. 57). The caudal vena cava passes through the cranial part of the right lobe close to its dorsal border (Figure 4.23).

The gall bladder is spindle-shaped (fusiform) and lies on the visceral surface of the right lobe. Each main liver lobe

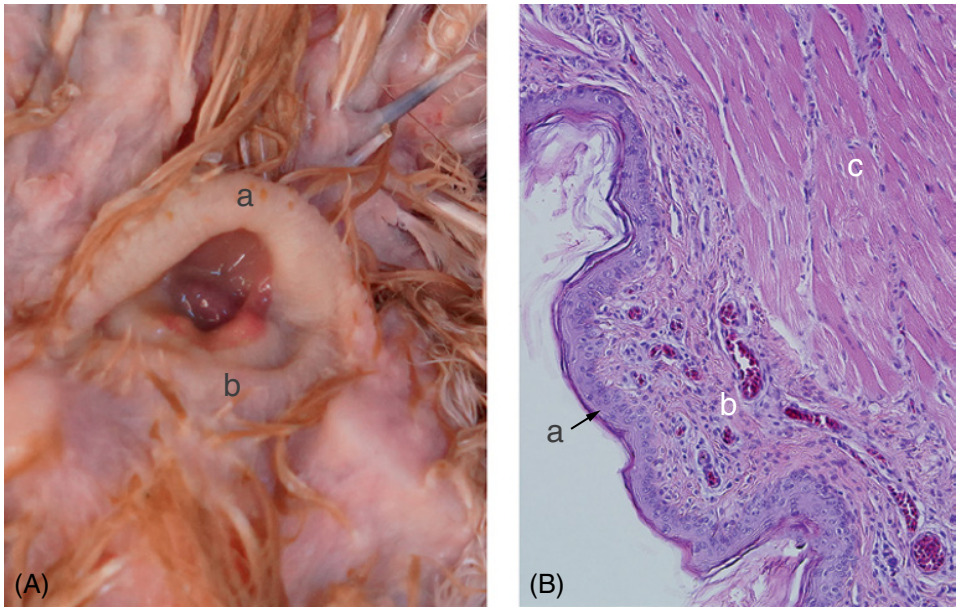

Figure 4.22 Vent of a female chicken images, (A) macroscopic and (B) histologic (H&E stain). (A) Dorsal (a) and ventral (b) lips of the vent. (B) Keratinized stratified squamous epithelium (a), dermis with highly vascularized dense irregular connective tissue (b), skeletal muscle bundles (c).

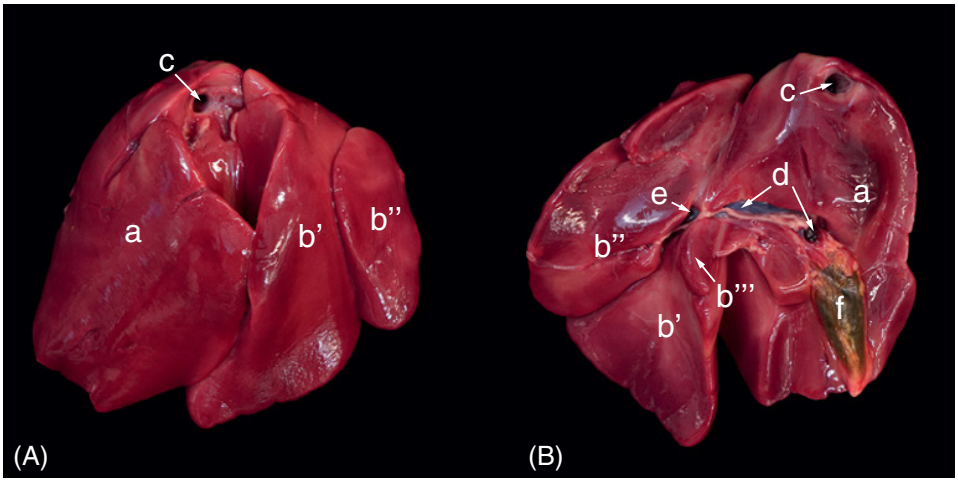

Figure 4.23 Parietal (A) and visceral (B) views of a chicken liver. (A) Right lobe (a); left lobe with medial (b'), lateral (b"), and intermediate (b"') portions; caudal vena cava (c); right (d) and left (e) hepatic portal veins; gall bladder (f).

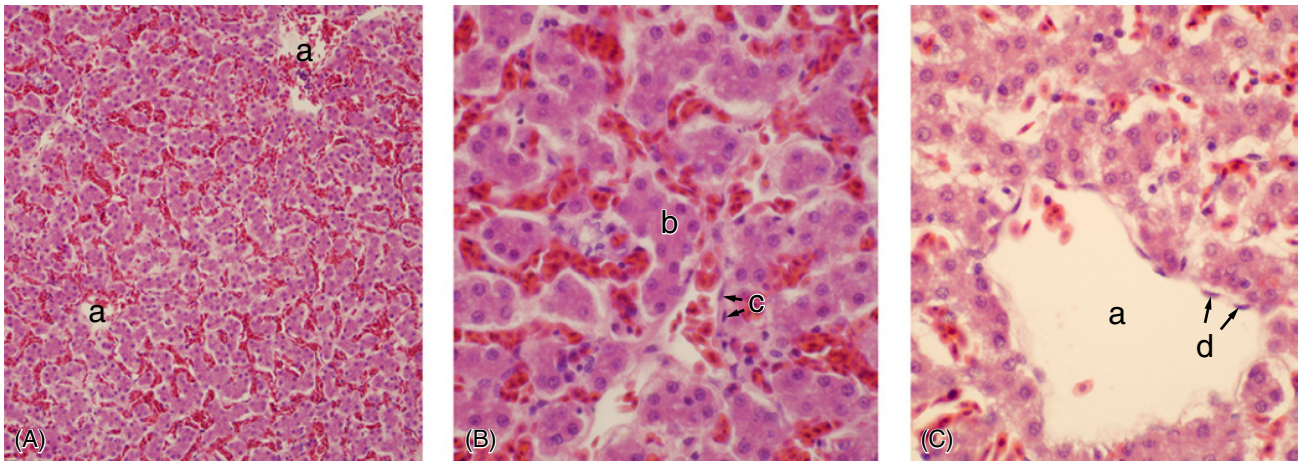

Figure 4.24 Histology of the chicken liver (A, B, and C). H&E stain. Central veins (a), double rows of hepatocytes (b), endothelial cells of the hepatic sinusoids (full of nucleated red blood cells) (c), and endothelial cells (d) of the central vein (a).

is drained by a bile duct. The hepatocystic duct drains bile from the right lobe to the gall bladder while the common hepatoenteric duct drains bile from both lobes to the duodenum. Histologically, the liver lobules are not well delineated, and each lobule is composed of hepatic cords arranged around a central vein (Aughey and Frye 2001; Eurell and Frappier 2006; Bacha and Wood 2012) (Figure 4.24). These cords are composed of double hepatocytes as opposed to single in mammals. The sinusoids are lined by endothelium (simple squamous epithelium). Kupffer cells are observed bulging into the lumen of the sinusoids. Triads (artery, vein, and bile duct) at the corners of the hepatic lobule are surrounded by connective tissue and infiltrated by large numbers of lymphocytes and plasma cells.

4.10.1 Hepatic Portal System and Blood Supply

As in mammals, the liver obtains blood from hepatic arteries and the portal veins and is drained by hepatic veins. The arterial blood supply to the liver is through the right and left hepatic arteries branching from the celiac artery. The fowl has two hepatic portal veins draining the gastrointestinal tract with its associated organs such as the spleen and pancreas. The left hepatic portal vein is small, restricted to a limited portion of left hepatic lobe and has the contribution from five tributary veins (the ventral proventricular, left proventricular, ventral gastric, left gastric, and pyloric veins) draining the proventriculus, gizzard, and pylorus. The right hepatic portal vein was the largest, receiving the

proventriculosplenic, gastropancreaticoduodenal, and common mesenteric veins then piercing the right hepatic lobe to be distributed in both hepatic segments through right and left divisions (Maher 2019). The liver is drained by two main large right and left hepatic veins and several smaller (middle and accessory) hepatic veins.

4.11 Pancreas

The pancreas is situated inside the loop of the duodenum within a fold of peritoneum called the duodenopancreatic fold (Figures 4.1 and 4.14). The dorsal and ventral lobes can be differentiated and a small segment, close to the spleen that is rich in islets of Langerhans, is called the splenic lobe. This lobe is very thin and embedded in adipose tissue. Both, the ventral and the dorsal lobes, are connected to each other. The splenic lobe joins the dorsal lobe. The pancreas has three ducts with the splenic lobe having no separate excretory duct. The pancreatic and bile ducts open into the ascending part of the duodenum opposite the cranial part of the muscular stomach. The exocrine portion is composed of a compound tubuloalveolar gland (Figure 4.25). The lobulations of the gland are not clear as they are in mammals because of the low quantity of connective tissue present separating these lobules. However, the lobation is obvious from the outside. The acini are lined by relatively high pyramidal to cuboidal cells. The base of the cells where the nuclei are situated stain darkly (basophilic) whereas the top of the cells where the secretory granules aggregate stain lightly (eosinophilic). These granules are considered the source of enzymes. During starvation, they occupy large portions of the cell, and some are secreted immediately after eating. Stellate shaped cells are observed in between the glandular acini. These cells differ in number from region to region. Centroacinar cells are also encountered. Flat simple squamous to simple cuboidal epithelium lines the intralobular ducts. These types of epithelia tend to increase in height toward the interlobar ducts. The intralobular ducts are fewer in number when compared to the mammalian pancreas. The stellate shape cells are dividing, and may replenish the basal cells of the acini of the pancreas. It seems that these cells are embryonal or have multipotent capabilities, which can divide to give basal cells or fibroblasts.

The endocrine portion of the pancreas is composed of the islets of Langerhans (pancreatic islets) (Figure 4.25). These islets are scattered circular structures embedded in the exocrine portion of the pancreas. They are usually surrounded by a thin layer of connective tissue. The cells inside the islets are arranged in the form of branching cords separated by sinusoidal capillaries. Several authors described two types of islets, alpha and beta. Alpha islets are larger and usually present within the pancreatic lobe at the junction of the ventral and dorsal lobes. These islets are also called the dark islets because they stain with argentaffin (argyrophilic staining). These islets have alpha and delta cells. Beta islets are scattered randomly in all pancreatic lobes. They are smaller than alpha islets and contain beta cells and a few delta cells. They are also called light islets because they do not take the argyrophilic stain.

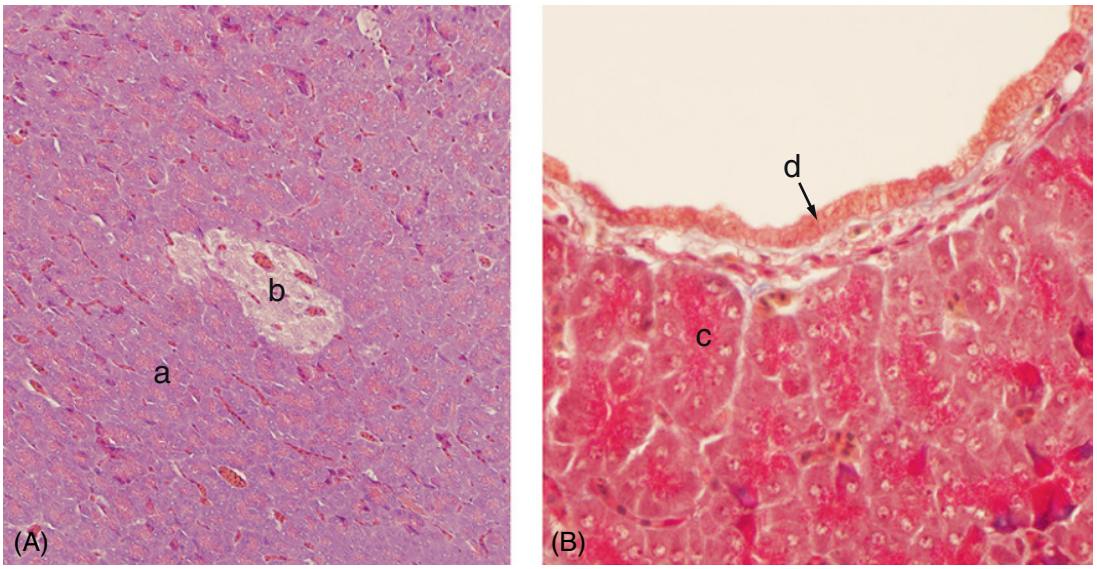

Figure 4.25 Histology of the chicken pancreas. (A) H&E (B) Trichrome stains. The exocrine portion of the pancreas (a) surrounds the small islet of Langerhans (b). The acini (c) are lined by pyramidal to cuboidal cells with nuclei located at the periphery and secretory granules aggregates toward the center. The large pancreatic ducts are lined by simple cuboidal epithelium (d).

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5

Respiratory System

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5.1 Introduction

The respiratory system of the chicken consists of the nasal cavities, reduced upper larynx (without vocal cords), lower larynx (syrinx) for phonation, trachea, two principal bronchi, lung, and air sacs. The nasal cavity has three nasal conchae (rostral, middle, and caudal) which open into the pharynx to communicate with the upper larynx. The relatively long trachea enables the chicken to breathe by preventing tracheal collapse while the bird moves its head and neck in a different directions. The air passes through the lung and to the caudal air sacs during inspiration and through the lung from the air sacs during expiration. Therefore, gaseous exchange in the lung takes place in both directions of the airflow. This allows for more efficient respiration than in mammals where gas exchange only occurs during inspiration.

Although the eggshell lacks ventilatory movements, it allows for limited respiration via simple diffusion of gases through the eggshell pores in the developing avian embryo (Tazawa and Whittow 2000). The major transition to lung respiration happens during the final days of embryonic life. The egg pores determine the developmental time of the respiratory system for the embryo (Ar and Rahn 1978) as well as the lung inflation time after hatching. In order for the gaseous exchange to take place, the accumulated fluid during the early stages of embryonic development needs to be removed during the first seven days after hatching from the lung tissue in order to allow for efficient gaseous exchange (Christensen 2009). The primary and secondary (mesobronchus) bronchi of the chicken lung are lined by a typical respiratory type of epithelium: ciliated pseudostratified columnar with goblet cells. Stratified squamous epithelium is encountered at the site of tracheal bifurcation into the two principal bronchi. A total of four cell types have been identified: ciliated, mucosecretory, basal, and endocrine. The presence of brush-like cells is reported by López et al. (2000).

5.2 Nostrils and Nasal Cavity

Nostrils or nares are supported by the underlying bony skeleton. Maxillary and frontal processes of the incisive (pre-maxilla) bone and nasal bone processes provide support to the nares (Casteleyn et al. 2018b). The opening of the external nares is supported by cartilaginous plates bound by the cornified operculum (König et al. 2016, p. 118), leaving the opening of the nares as an inverted sickle shape (Casteleyn et al. 2018b).

Past the nostrils, the nasal cavity is continued caudally by the nasal vestibule which opens into the nasal cavity proper, where the major structures observed are the nasal conchae (turbinates). They are thin scrolls of bone that occupy most of the nasal cavity, which are arranged rostrocaudally in contrast to mammals where they are arranged dorsoventrally (Figures 5.1–5.3). Their names are rostral, middle (ventral, inferior), and caudal (dorsal, superior) nasal conchae (Kırbaş Dogan and Takci 2018; Dyce et al. 2010, p. 799). While the nasal vestibule and rostral concha are lined with stratified squamous epithelium (non-glandular), middle conchae are lined with respiratory epithelium (ciliated pseudostratified columnar with goblet cells) (Figure 5.3) and finally, caudal concha is lined with olfactory epithelium (Eurell and Frappier 2006, p. 167; Kırbaş Dogan and Takci 2018). The nasal septum subdivides the nasal cavity into left and right halves utill the opening of choana, which communicates caudally with the oropharynx (Dyce et al. 2010, p. 799). The ventral nasal meatus, ventral nasal septum, and choanal openings carry

Anatomy and Histology of the Domestic Chicken, First Edition. Edited by Wael Khamas and Josep Rutllant.

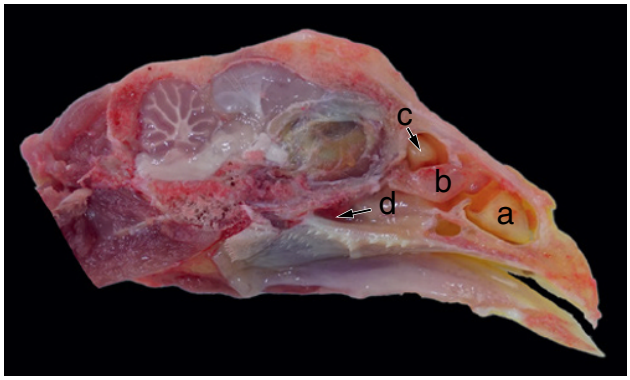

Figure 5.1 A sagittal section of a chicken head showing the nasal cavity. (a) Rostral concha, (b) middle concha, (c) caudal concha, and (d) choana.

nasal-associated lymphoid tissue in the mucosa. Many lymphocytes are distributed as diffuse lymphoid tissues in the submucosa, under the epithelium of the middle nasal concha and the walls of the nasal cavity (Kang et al. 2013). The presence of diffuse lymphatic tissues in the conchae of the nasal mucosa acts as the first line of defense and initiates an immune response to antigens. Both T, specifically CD3+ T lymphocytes, and B cells are identified in the chicken nasal mucosa (Nochi et al. 2018; Palanisamy et al. 2020). The presence of lymphatic tissue in the nasal cavity facilitates the immune response when the vaccine is administered intranasally (Sato and Kiyono 2012).

The infraorbital sinus is a space cranioventral to the eye that communicates with the nasal cavity via the caudal concha (Dyce et al. 2010, p. 800; Casteleyn et al. 2018b;

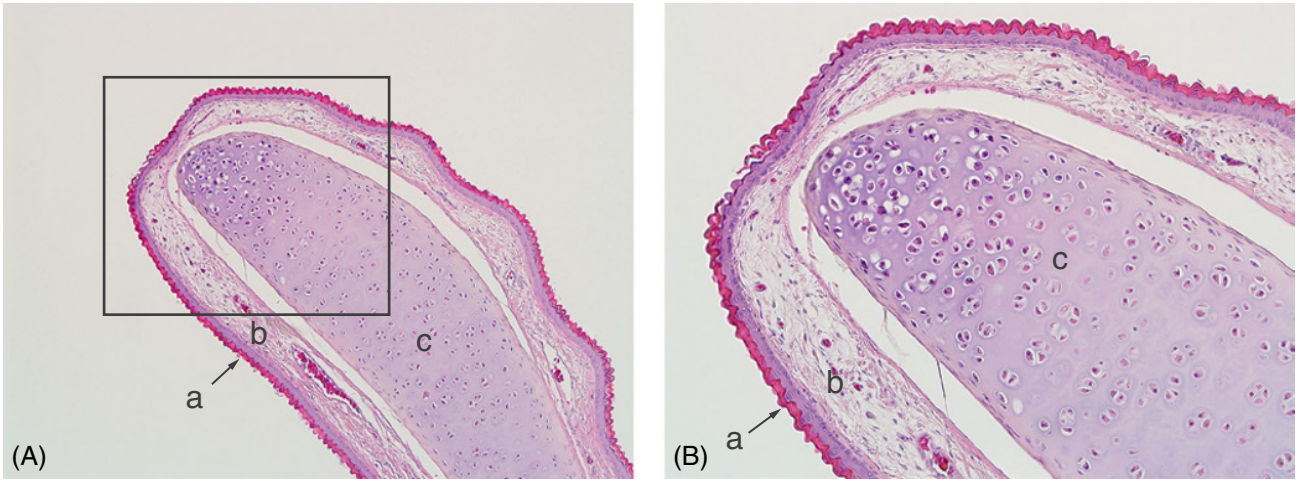

Figure 5.2 (A and B) Histological section of the rostral concha of the nasal cavity of the chicken. H&E stain. (a) Stratified squamous epithelium, (b) lamina propria/propria submucosa, and (c) hyaline cartilage.

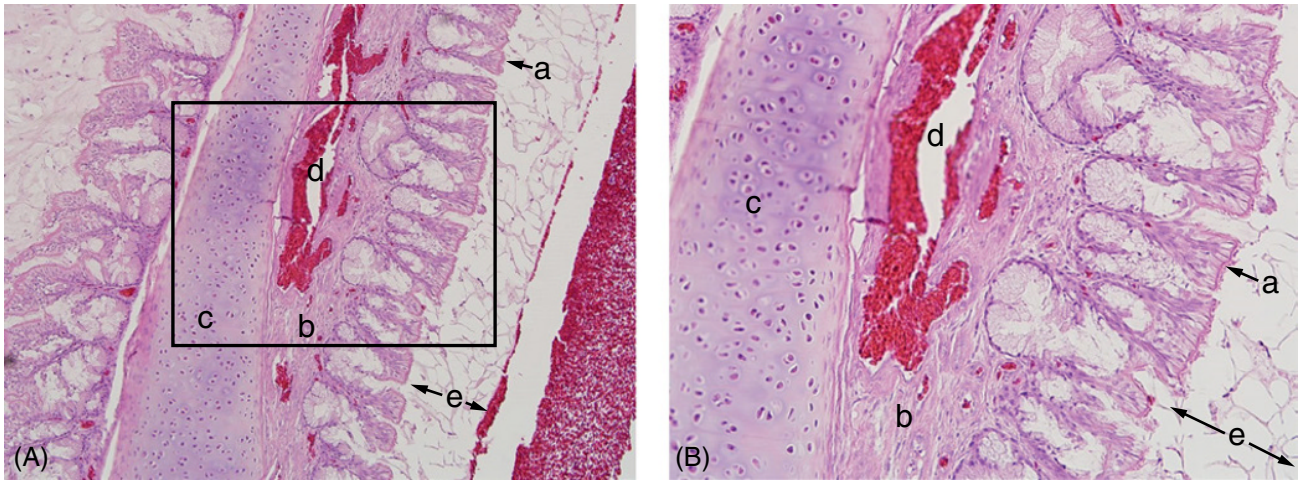

Figure 5.3 (A and B) Histological section of the middle nasal concha. H&E (a) Respiratory epithelium (pseudostratified ciliated columnar), (b) lamina propria/submucosa, (c) hyaline cartilage, (d) venous sinuses, and (e) mucus blanket.

Nickel et al. 1977, p. 63; Baumel et al. 1993, p. 264). This sinus is lined with respiratory epithelium and it has multiple recesses in the head region (Kırbaş Dogan and Takci 2018). Ventral to the border of the rostral and middle concha, the nasolacrimal duct has its opening into the nasal cavity (König et al. 2016, p. 119).

5.3 Larynx (Larynx Cranialis), Upper Larynx

The larynx has a mound, or mons laryngealis, which is an elevated bulge that has the glottis aiming dorsally. It is situated in the ventral aspect of the oropharynx and carries an opening, aditus laryngis, bordered by the left and right glottis. In contrast to the mammalian larynx, it lacks epiglottic and thyroid cartilages. Instead, only a circular cricoid, a single procricoid (Kırbaş Dogan and Takci 2018; Baumel et al. 1993, p. 266), and paired arytenoid cartilages are present which provide the structural support to the soft laryngeal tissues (Figure 5.4). The arytenoids support the glottis which carries a slanted slit named the glottic cleft. The procricoid is positioned in the dorsomedian aspect, thus articulating with the cricoid and the arytenoids (Figures 5.4–5.6) (Kırbaş Dogan and Takci 2018; Baumel et al. 1993, p. 266). Procricoid cartilage articulates with the two arytenoid cartilages via synovial joints (Figure 5.6). All articulations between the laryngeal cartilages are true synovial joints (Kırbaş Dogan and Takci 2018). Inner muscles in the larynx are roughly separated into laryngeal constrictors and dilatators. Dilator muscle of the glottis is located under the mucosa in between the cricoid and the arytenoid cartilages (Kırbaş Dogan and Takci 2018). The constrictor muscle of the glottis originates from the procricoid cartilage and inserts into both the cricoid and arytenoid cartilages in a horseshoe shape (Kırbaş Dogan and Takci 2018). The vocal folds (cords) are absent; therefore, sound production is not occurring in the upper larynx as is the case in mammals. A major function of the larynx is to prevent the aspiration of foreign particles and liquids into the respiratory system (König et al. 2016, p. 121). The mucosal lining of the larynx is ciliated pseudostratified columnar epithelium with goblet cells (Eurell and Frappier 2006, p. 167). Goblet and ciliated cells are dispersed throughout the laryngeal surface. There are large clusters of pear-shaped intraepithelial multicellular mucous-secreting glands (Figure 5.5). The lamina propria is thin and is fused with the perichondrium of the laryngeal cartilages. In the dorsal aspect of the larynx, peripheral to the laryngeal cartilages, large skeletal muscles

Figure 5.5 Histological section of the caudal part of the upper larynx of the chicken. H&E (a) Body of the cricoid cartilage, (b) mucosa of the larynx, (c) mucosal associated lymphoid tissue, (d) mucus glands, and (e) tracheolateralis muscle.

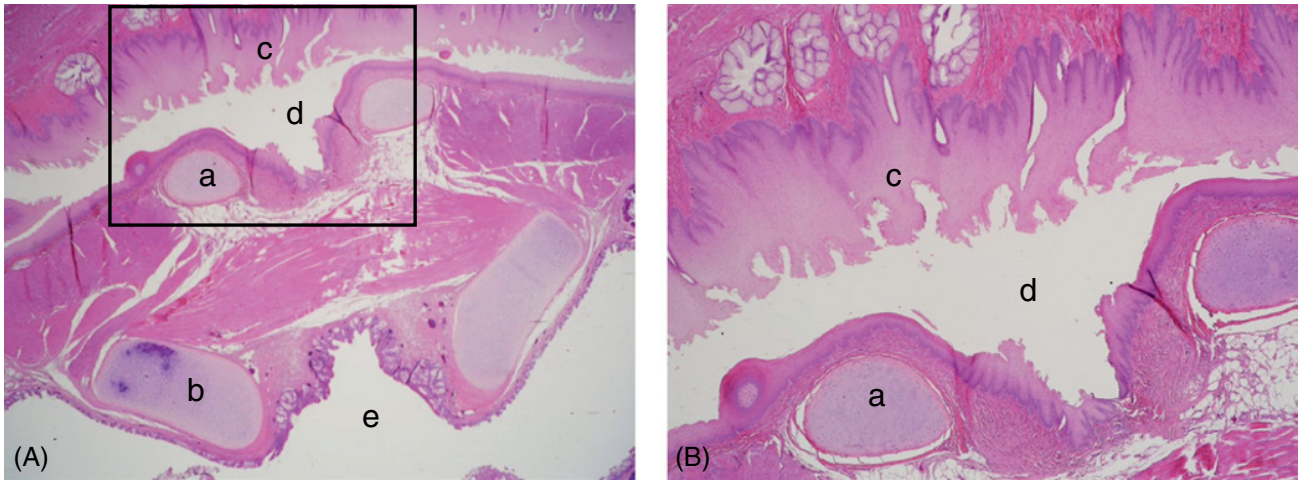

Figure 5.4 (A and B) Histological section of the rostral part of upper larynx of the chicken. H&E (a) Caudal thin part of arytenoid cartilage, (b) body of arytenoid cartilage, (c) mucosa of oropharynx, (d) lumen of oropharynx, and (e) lumen of larynx.

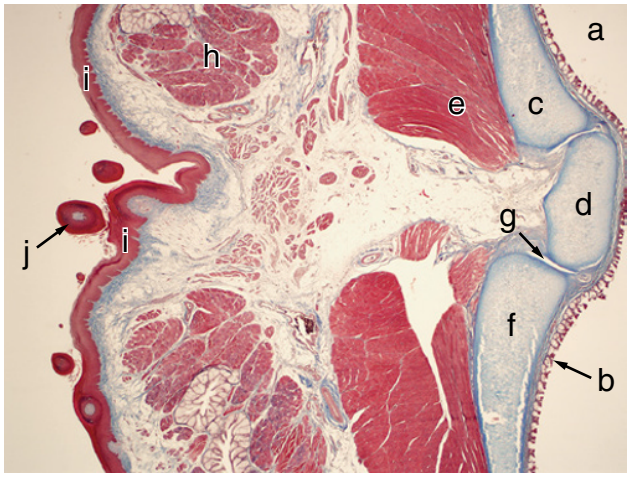

Figure 5.6 Histological section of the upper larynx. Trichrome (a) Laryngeal lumen, (b) laryngeal epithelium, (c) cricoid cartilage wing, (d) procricoid cartilage, (e) constrictor muscle of the glottis, (f) cricoid cartilage wing, (g) procricoid-cricoid synovial joint, (h) dilator of the glottis muscle, (i) outer mucosal of the laryngeal mound, stratified squamous epithelium, and (j) cornified papilla (cross-section).

(tracheolateralis) are observed inserted into the cartilages. In addition, there is diffuse lymphatic tissue and more organized lymph nodules are present in the lamina propria submucosa of the larynx. Its lamina propria submucosa has large clusters of mucous glands and dispersed skeletal muscles which are associated with the laryngeal cartilages.

The laryngeal mound from outside is covered with cornified (keratinized) stratified squamous epithelium and carries two rows of cornified papillae that project in the caudal direction.

5.4 Trachea

The trachea is a tubular organ that originates at the caudal aspect of the cricoid cartilage of the larynx. It courses through the neck ventral midline, in the beginning, and more caudally it diverges more to the right side where it is closely associated with the esophagus. Structural support to the trachea is provided by cartilaginous rings (Mennega 1964), which decrease in diameter closer to the thoracoabdominal cavity. The trachea is composed of complete hyaline cartilage rings, which often overlap with the adjacent rings and are connected with each other by loose connective tissue. The cartilages have thick and thin portions which result in adjacent cartilages interdigitate with each other. The thin portion of one cartilage enters inside the thick portion of the adjacent cartilage. While inside the larger rings, the smaller rings result in the formation of what appears as "two tubes" but is actually the same tube overlapping on itself (Figure 5.7A). The mucosal lining of the trachea is a respiratory epithelium: ciliated pseudostratified columnar with goblet cells (Eurell and Frappier 2006, p. 167). There are large clusters of multicellular intraepithelial pear-shaped mucous-secreting glands which communicate freely with the lumen (Figure 5.7A and B). Beneath the epithelium, there is a very thin lamina propria comprised of loose connective tissue with blood capillaries. The lamina propria attaches to the perichondrium of the tracheal rings.

Peripheral to the cartilaginous tracheal rings, there is a layer of connective tissue or adventitia, and sometimes strong longitudinal skeletal muscle bundles (latero trachealis muscle) (Figure 5.7A and B). The muscle connective

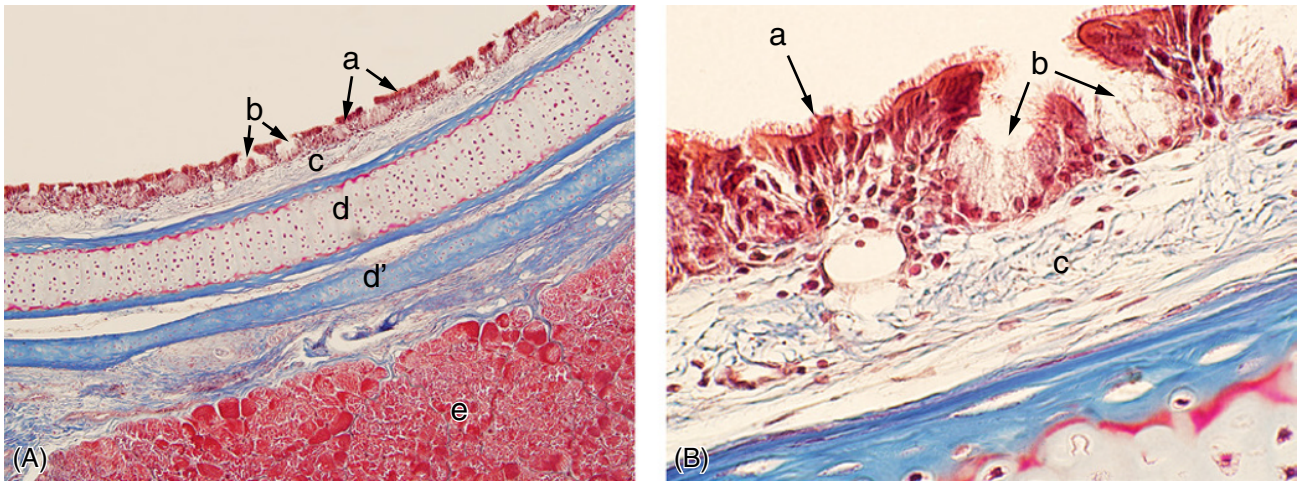

Figure 5.7 (A and B) Histological section of the trachea of a chicken. Trichrome (a) Respiratory epithelium of the trachea, (b) multicellular mucous glands, (c) lamina propria/propria submucosa, (d) tracheal cartilages (hyaline), (d') overlapping portion of adjacent cartilagenous ring, and (e) tracheolateralis muscle.

tissue bundle's epimysium is fused with the perichondrium of the tracheal rings. The skeletal muscles anatomically and functionally related to the trachea are sternotrachealis, cleidotrachealis, laterotrachealis, and cleidohyoideus (Casteleyn et al. 2018b). Sternotracheal muscle originates from the sternum and inserts at the trachea cranial to the syrinx at the level of the coracoid bone (Casteleyn et al. 2018b; Kırbaş Dogan and Takci 2018). Cleidotrachealis muscle originates from the clavicle and inserts at the trachea cranial to the insertion site of the sternotrachealis (Kırbaş Dogan and Takci 2018; König et al. 2016, p. 121). Tracheolateralis muscle originates at the syrinx and continues to the cricoid cartilage (Casteleyn et al. 2018b). Sternohyoid muscle originates from the sternum and courses to the larynx and the trachea (Kırbaş Dogan and Takci 2018). Stimulation of the caudal part of the tracheolateralis muscle draws the tympanum cranially, the external tympaniform membranes were stretched, thus the syringeal lumen enlarged. Stimulation of the sternotrachealis muscles produces syrinx constriction. Stimulation of the cranial parts of both tracheolateralis and tracheohyoideus muscles produced a marked retraction of the larynx and rostral part of the trachea (Brackenbury 1980).

5.5 Syrinx (Larynx Caudalis), Lower Larynx

The syrinx is found in between the caudal end of the trachea and the beginning of the paired principal bronchi (Figure 5.8). The tracheal rings (tracheosyringeal cartilages) at the end of the trachea are complete cartilaginous rings. The principal bronchi have incomplete cartilaginous rings (bronchosyringeal cartilages) at their origin (Baumel et al. 1993, pp. 291–297).

Roughly, the last 3–4 complete tracheal cartilaginous rings create a tympanum in chicken (Kırbaş Dogan and Takci 2018) which forms the beginning of the syrinx. Caudally, there is a median bone, pessulus (cartilaginous in some birds), which marks the opening and the bifurcation to both principal bronchi (Figure 5.8). The bony pessulus helps the medial tympaniform membranes to be stiffer and to vibrate strongly so that a louder sound will be generated. The pessulus is cranially domed and its periphery is lined by pseudostratified columnar epithelium with intraepithelial goblet cells and a few simple alveolar glands.

The very origin of the principal bronchi is membranous. Their medial and lateral walls are referred to as medial and lateral tympaniform membranes. Both medial and lateral tympaniform membranes are used in vocalization (phonation) in chicken. Lateral tympaniform membranes are connected to the C-shaped cartilages (incomplete rings) of the

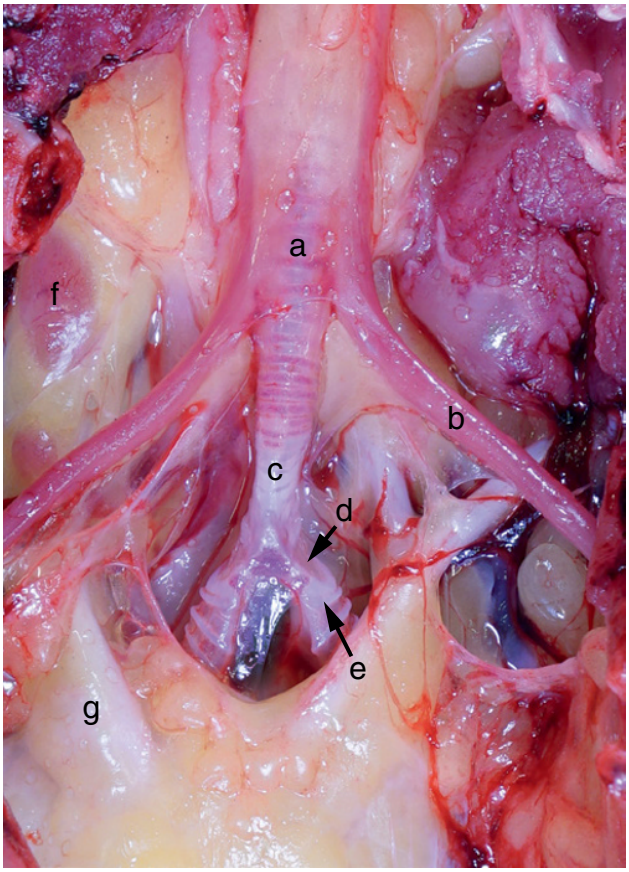

Figure 5.8 Syrinx and surrounding structures of a chicken. (a) Trachea, (b) sternotrachealis muscle, (c) ossified cartilages of the trachea, (d) lateral tympanum, (e) ossified cartilages of the left principal bronchus, (f) thyroid gland, and (g) right brachiocephalic trunk.

principal bronchi, which are present laterally. The external (lateral) tympaniform membranes are proved through experimentation of cutting them as the essential vocal structures of the chicken (Gross 1964). Tympaniform membranes are lined with stratified squamous epithelium (Eurell and Frappier 2006, p. 168) in contrast to the rest of the trachea and the bronchi. Syrinx has intrinsic muscles that aid in phonation and extrinsic syringeal muscles, which also include some tracheal muscles; hence, both tracheal and syringeal muscles control phonation in birds (Casteleyn et al. 2018b). The sound generated within the syrinx is a coordinated effort of cartilages, muscles, and membranes. The pressure generated by the air passage during exhalation affects the tympanic membranes accompanied by contraction and relaxation of adjacent muscles, resulting in the generation of sound (Goller and Larsen 1999). Moreover, Daley and Goller (2004) stated that resonance in the upper vocal (respiratory) tract is also thought to affect sounds generated in the syrinx, similar to the process that occurs in humans within the larynx.

Electron microscopic examination of the syringes of the fowls and pigeons revealed numerous immune cells, including dendritic, plasma, mast cells, and lymphocytes distributed within the syringeal mucosa and scattered through the syringeal epithelium (Ibrahim et al. 2020).

5.6 Lungs

The lung of the chicken is confined to the craniodorsal aspect of the thoracoabdominal cavity. The chicken lungs are small, firm to touch, and bright pink in color in comparison to mammalian lungs (Figure 5.9) (Dyce et al. 2010, p. 802). Dorsally, they are firmly anchored to the ribs and the vertebrae by connective tissue. As a result of this interaction, they are carrying deep grooves or impressions (sulci costales) on the dorsal surface (Figure 5.9). The lungs do not encompass the lateral aspect of the heart indicating their smaller size compared to the mammalian lungs. The ventral aspect of the lung carries a pulmonary hilus marking the site where the principal bronchi and pulmonary vessels merge with the pulmonary parenchyma. In addition, the ventral surface is connected to the thin membrane the horizontal septum (König et al. 2016, p. 124). The cranial and caudal aspects of the ventral surface of the lungs have saccobronchial connections to the air sacs, which are functionally important for airflow efficiency in poultry (Figures 5.10–5.12). The capacity of the lungs to expand is negligible due to its high cartilaginous content (Dyce et al. 2010, p. 802). However, apart from the principal bronchi, small nests of cartilage were detected in the interstitium of the terminal divisions of the air passages of chickens of different ages. They were more frequently present in young chicken which suggested that this is a dysontogenetic feature (Kaliner 1976). As the principal bronchus enters the lung parenchyma, it continues horizontally and connects directly to the abdominal air sac (König et al. 2016, p. 125; Dyce et al. 2010, p. 802) (Figure 5.10). As it progresses

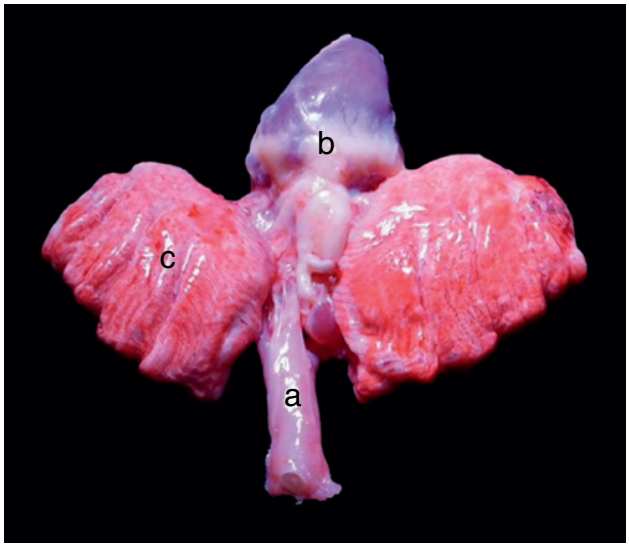

Figure 5.9 Chicken lungs, dorsal view. (a) Trachea, (b) heart, and (c) right lung with visible sulci costales (rib impressions).

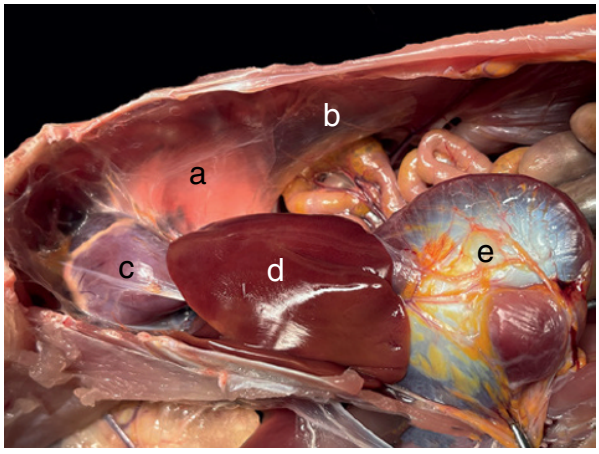

Figure 5.11 Chicken thoracoabdominal cavity, upper part of the image is ventral and left side of image is cranial. (a) Cranial thoracic air sac, (b) caudal thoracic air sac, (c) heart, (d) liver, and (e) muscular stomach (gizzard).

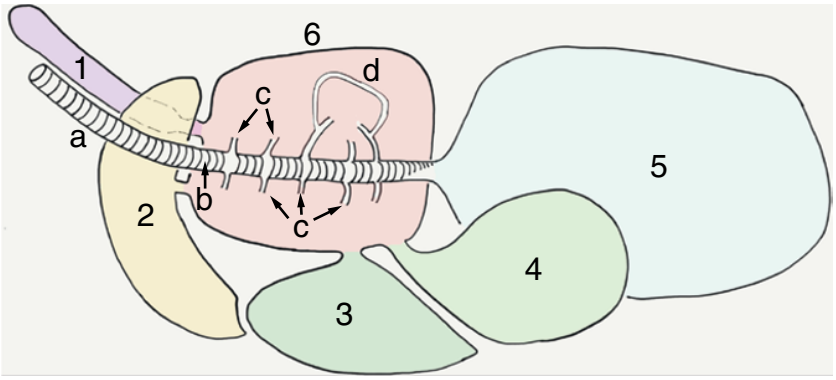

Figure 5.10 A diagrammatic representation of the trachea and air sacs of a chicken. (1) Cervical air sac, (2) clavicular air sac, (3) cranial thoracic air sac, (4) caudal thoracic air sac, (5) abdominal air sac, (6) lung, (a) trachea, (b) primary bronchus, (c) secondary bronchus, and (d) parabronchus.

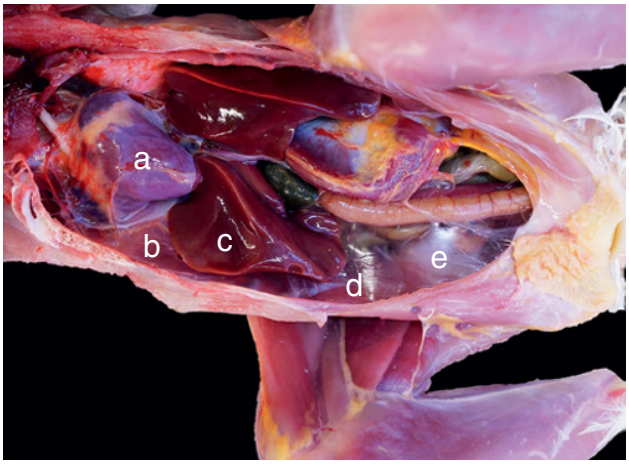

Figure 5.12 Chicken thoracoabdominal cavity, lower part of the image is ventral and left side of the image is cranial. (a) Heart, (b) cranial thoracic air sac, (c) liver, (d) caudal thoracic air sac, and (e) abdominal air sac.

Figure 5.14 Histological section of the lung parenchyma, parabronchus. P.A.S. stain (a) Interparabronchial septum, (b) infundibulum, (c) atrium, (d) lumen of the parabronchus, (e) cuboidal epithelium, (f) air capillaries interwind with blood capillaries, (g) fused interbronchial tissue, and (h) a branch of pulmonary vessel.

caudally, it gives off 40–50 secondary bronchi (Dyce et al. 2010, p. 802) (Figure 5.10). These diverge in medioventral, mediodorsal, lateroventral, and laterodorsal directions and are named accordingly (Dyce et al. 2010, p. 802). Secondary bronchi have also established connections with the air sacs, which are crucial to the function of the airflow during respiration. Secondary bronchi give off 400–500 parabronchi (Dyce et al. 2010, p. 803) (Figures 5.10, 5.13A and B, 5.14, and 5.15A and B), which constitute the bulk of the pulmonary parenchyma and provide the connection to the sites for gaseous exchange. Parabronchi are the equivalent of the segmental/tertiary bronchi in mammals. Parabronchi commencing from medioventral and mediodorsal secondary bronchi, which connect to each other, constitute paleopulmo (old lung), and the ones emerging from lateroventral and laterodorsal give rise to neopulmo (new lung). The paleopulmo is the larger portion of the lung and it accounts for approximately 75% of the lung volume in most bird species (King and McLelland 1975 p. 51; Casteleyn et al. 2018a), but the difference between the two compartments, paleopulmo and neopulmo, is merely functional and cannot be differentiated macroscopically.

The secondary bronchi and parabronchi also anastomose with each other in the vicinity, via the functional division of the neopulmo (Dyce et al. 2010, p. 803). However, for the most part, they are separated from each other by interparabronchial septae (connective tissue) (Figures 5.14 and 5.15A and B) (Nickel et al. 1977, p. 66). Interparabronchial septae

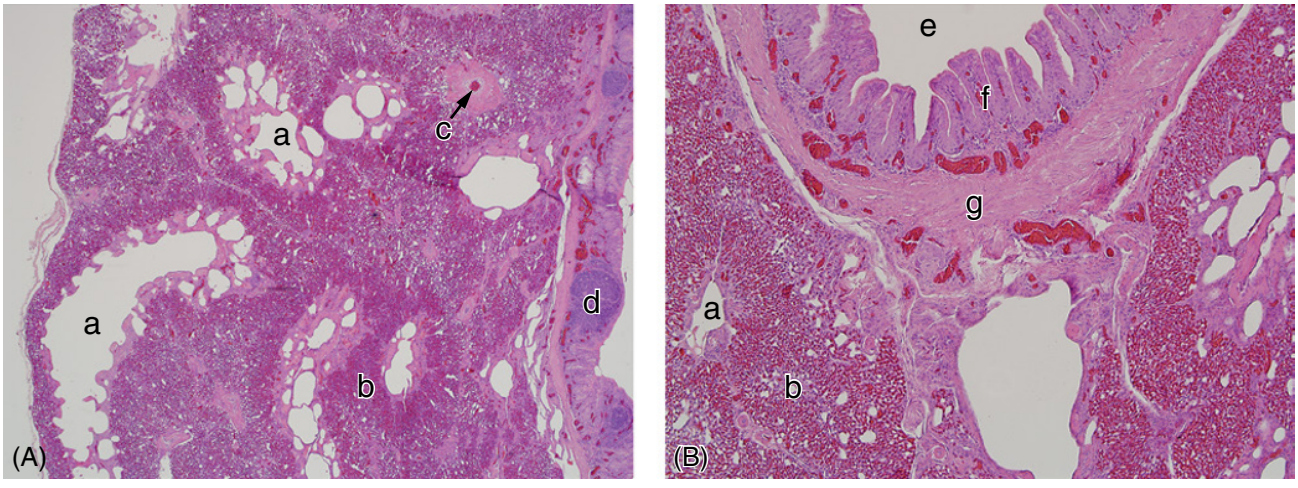

Figure 5.13 (A and B) Histological section (H&E stain) of the lung histology section showing: (a) parabronchi lumen surrounded by (b) air and blood capillaries, (c) pulmonary artery, (d) bronchial associated lymphoid tissue, (e) secondary bronchus, (f) high epithelium (pseudostratified columnar with thick muscular wall), and (g) smooth muscle.

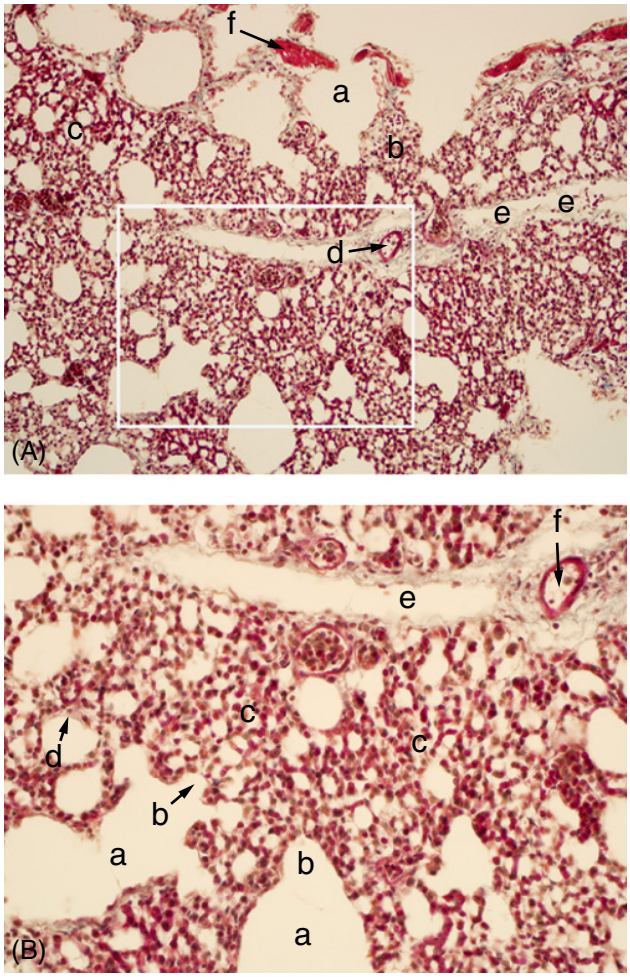

Figure 5.15 (A) Histological section of the lung parenchyma and parabronchus. P.A.S. stain. (a) Atrium, (b) infundibulum, (c) air capillaries, intertwined with blood capillaries, (d) larger blood vessels, (e) interparabronchial septum, and (f) smooth muscle bundles covered by simple cuboidal epithelium.
(B) Parabronchus functional parenchyma. (a) Atrium, (b) infundibulum, (c) air capillaries, intertwined with blood capillaries, (d) air capillary lining epithelial cell, (e) interparabronchial septum, and (f) larger blood vessels.

serve as passageways for larger blood vessels (arteries and veins) on their way to the functional parenchyma of the lungs (Figures 5.13A and B, 5.14, and 5.15A and B) (König et al. 2016, p. 125). Parabronchi are roughly hexagonal in transverse section and have 1 to 2 mm diameter in chicken (Dyce et al. 2010, p. 803; Nickel et al. 1977, p. 67; Eurell and Frappier 2006, p. 168). Centrally, they have a lumen and their walls are carrying functional respiratory units. From the central lumen of the parabronchus, the atria emerge as small evaginations, which funnel into infundibula and subsequently give rise to the tubules, called air capillaries. Air capillaries intermingled with blood capillaries form the functional parenchyma of the bird's lung and are equivalent to the alveoli in mammals. Air capillaries and interlaced blood capillaries constitute a major part of the parabronchial wall and the lung parenchyma. Principal and secondary bronchi are similar in histological structure to the trachea, and are lined with respiratory epithelium; however, cartilages are progressively becoming smaller, incomplete, and patchy (Eurell and Frappier 2006, p. 168). The epithelium lining the lumen of the parabronchi is a simple cuboidal to simple squamous. Tips of the atria are carrying simple squamous epithelium and are structurally reinforced by bundles of smooth muscle cells in the lamina propria (Eurell and Frappier 2006, p. 168). Air capillaries are continuing with the atria and are 3–15 µm in diameter. The gas exchange areas are lined with the simple squamous epithelium and has both pneumocyte type I and pneumocyte type II cells, which is consistent with the mammalian lungs. A biphasic layer secretion by pneumocyte type II is present through the secretion of the surfactant (Eurell and Frappier 2006, p. 168). The flow of the air in the air capillaries is crosscurrent (Dyce et al. 2010, p. 803) which contributes to the high efficiency of the chicken respiratory system. Instead of being the terminal end of the respiratory system, such as alveoli in mammals, air capillaries are continuous tubes in which gaseous exchange occurs continuously, regardless of the direction of the air. Brush cells were found within the bronchial epithelium, but never within the gas exchange areas. Their numbers are extremely low, scattered among the other epithelial cells, and mainly ciliated. The morphologic characteristic of these cells is apical protrusions of the cytoplasm with microvilli. The functional role of these cells is not clear, though they may have an absorptive function according to López et al. (2000).

The flow of the air through lung parenchyma (air capillaries) is always in one direction and the flow of the blood in blood capillaries is also in one direction, which in fact is the opposite direction to the flowing air. This is referred to as crosscurrent aka countercurrent flow. This feature gives exceptional efficacy for gas exchange at the level of the blood-gas barrier.

5.7 Direction of Airflow

Air flows in one direction in the lungs of the birds. The air sacs permit a unidirectional flow of air through the lungs to exchange oxygen during inhalation and exhalation. Birds can breathe easier at higher elevations compared to

mammals because of the efficiency of their respiratory system. In birds, unlike in mammals, both inspiration and expiration are active processes that require muscular activity. During inspiration, the sternum moves cranially and ventrally while the vertebral ribs move cranially to expand the sternal ribs and the thoracoabdominal cavity. The uncinate processes in birds are important structures through facilitating the movements of the ribs and sternum during breathing (Codd et al. 2005). The respiratory muscles' actions generate forces that are required to move gas through the air sacs and the lungs (Codd et al. 2005). This expands the caudal and cranial air sacs and lowers the pressure, causing air to move into those air sacs. Air from the trachea and bronchi moves into the caudal air sacs and, simultaneously air from the lungs moves into the cranial air sacs. Gas that reaches the cranial air sacs must pass through the paleopulmonic parabronchi air capillaries and exchange gases with the blood capillaries surrounding them. Air that moves through the paleopulmonic parabronchi, the main gas-exchanging bronchi, in the lung is in the same direction during both inspiration and expiration. During expiration, airway resistance is increased in the intrapulmonary primary bronchus because of the dynamic compression causing the air to enter the mediodorsal secondary bronchi. To understand the flow of air during inspiratory and expiratory events observe diagram (Figure 5.16).

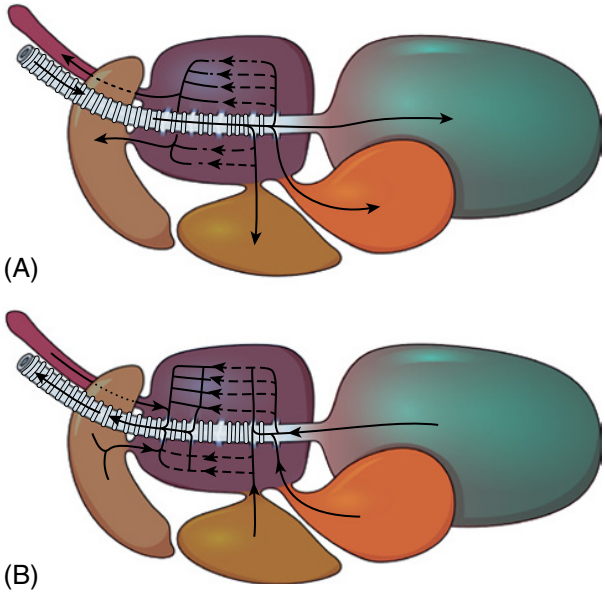

Figure 5.16 Diagrams of the trachea, air sacs, and the direction of airflow during (A) inspiration and (B) expiration.

5.8 Blood-Gas Barrier

The avian blood-gas respiratory tissue barrier is 56–67% thinner than that of the mammalian lung, however, equivalent body weight and the respiratory surface is 15% greater (Maina et al. 1982). The thin gaseous exchange barrier also facilitates the passage of foreign particles, because of its weak first line of defense, the incidence of the introduction of pathogens into the avian respiratory system is higher than in mammals. It has been demonstrated that the free avian respiratory macrophage population increases following the transmigration of the cells from the avian lung subepithelium, or the vascular system, in response to invading foreign particles and pathogens. The specific macrophage defense activity though is not fully understood yet. It is postulated that the presence of more efficient lysosomal activities results in an efficient cytotoxic effect on the pathogens (Nganpiep and Maina 2002; Toth and Siegel 1986).

5.9 Air Sacs

The air sacs are thin, translucent membranes lined by simple squamous to simple cuboidal epithelium. The only exceptions are the small regions at the junction with primary and secondary bronchi where the lining transitions to respiratory epithelium (Eurell and Frappier 2006, p. 168). Air sacs connect to primary and secondary bronchi. The air sacs are functionally viewed as extensions of the airways (bronchi) and are not regarded as gas exchange membranes (Kırbaş Dogan and Takci 2018). Air sacs, in general, are poorly vascularized and therefore gaseous exchange is not possible (Magnussen et al. 1976). Clinically, air sacs decrease the uptake of inhalant anesthetics and allow for their accumulation (Christensen et al. 1987).

Chicken has eight air sacs: single clavicular and cervical and paired cranial thoracic, caudal thoracic, and abdominal. Air sacs occupy spaces between different organs and can be distinguished into two distinct functional groups: cranial (cervical, clavicular, and cranial thoracic) and caudal (caudal thoracic and abdominal) air sacs (Figure 5.10). Some air sacs form diverticula which pneumatize bones (Kırbaş Dogan and Takci 2018). The cervical sac directly connects to the first medioventral secondary bronchus. The cervical air sac has a chamber positioned at the midline and laterally it has two diverticula (Dyce et al. 2010, p. 803; Nickel et al. 1977, p. 68) which pneumatize cervical and thoracic vertebrae, by entering their transverse foramina and the vertebral canal. The clavicular air sac directly connects to the third medioventral secondary bronchus

and may also have indirect connections via parabronchi to other cranial (medioventral) secondary bronchi in some species. Clavicular air sac also pneumatizes the humerus (extrathoracic diverticulum) and the sternum (thoracic diverticulum). Cranial thoracic air sacs are paired and positioned ventral to the lungs, bordered by the heart, liver, and sternal ribs (Dyce et al. 2010, p. 803; Kırbaş Dogan and Takci 2018). The cranial thoracic air sacs connect to the third medioventral secondary bronchi and also to parabronchi originating from other cranial secondary bronchi in some species.

Paired caudal thoracic air sacs are more caudally positioned alongside the thoracoabdominal cavity and are caudally delimited by the abdominal air sacs. They are directly connected to the lateroventral secondary bronchus and may have indirect connections to other lateroventral or even cranial (medioventral) secondary bronchi in chicken with large amounts of neopulmonic parabronchi. The paired abdominal air sacs are the largest and occupy the caudodorsal part of the thoracoabdominal cavity apposing the abdominal viscera. They have diverticula that pneumatize the acetabulum and the synsacrum (Dyce et al. 2010, p. 803; Nickel et al. 1977, p. 69; Casteleyn et al. 2018a). The abdominal air sacs connect to the caudal end of the intrapulmonary principal bronchus and may have more indirect connections to parabronchi from laterodorsal secondary bronchi and the last mediodorsal secondary bronchi. Air sac connections with parabronchi are frequently grouped into a funnel-like structure called the saccobronchus.

The existence of air sacs is what makes the avian respiratory tract more efficient in comparison to mammals as the air percolates through lung parenchyma during both inspiration and expiration. The cranial functional group is mainly connected to the ventral secondary bronchi, whereas the abdominal air sac (caudal functional group) is connected via principal (primary) bronchi (Figure 5.16). The cranial functional group upon inspiration receives the air that is already depleted from oxygen since it has passed through lung parenchyma (parabronchi). However, the caudal functional group contains relatively fresh air that has passed mostly directly through the principal bronchus and its oxygen content is not depleted. Upon expiration that fresh air is being pushed into the lung and passes through the lung functional parenchyma (neopulmo, parabronchi). Concurrently, the air from the cranial functional group in expiration is pushed mostly to the trachea (König et al. 2016, p. 128). In birds, unlike in mammals, both inspiration and expiration are active processes that require muscular activity. The movement of the air through the lung and air sacs is facilitated by the movement of the sternum and ribs (Dyce et al. 2010, p. 803), and the ribs are pushed in cranial and the sternum is pushed ventrally. During contraction of the inspiratory muscles, there will be an increase in thoracoabdominal volume. Thus, volume changes only take place in the air sacs (Scheid and Piiper 1989). During inspiration, pressure within the air sacs becomes negative relative to the ambient atmospheric pressure, and air flows from the atmosphere into the air sacs and across the gas exchange surfaces of the lungs. The major muscles for inspiration are external intercostals and costosternalis, whereas the main expiratory muscles are internal intercostal and abdominal muscles (Dyce et al. 2010, p. 804).

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6

Urinary System

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6.1 Introduction

6.1.1 Overall Composition and Function of the Urinary System

The avian urinary system presents unique features when compared to the homologous system in mammals. Birds do not have renal pelvis, urinary bladder, or urethra. Regarding internal organs in birds, there is a noticeable reduction in the size of the kidneys, which plays a role in the overall weight reduction that facilitates the capability of flight (Nickel et al. 1977, pp. 70–72). Both kidneys are partially lobated and they are located immediately behind the lungs and on both sides of the vertebral column. The ureters are located ventromedially to the kidneys, leading to the urodeum part of the cloaca.

As in mammals, the functional unit of the avian kidney is the nephron. It consists of the renal glomeruli, the Bowman's capsule (both together form the Malpighian/renal corpuscle), the proximal and distal convoluted tubules, and thin limb within the medulla (like Henle's loop for the mammalian nephrons). Only a reduced number of mammalian-like nephrons with Henle's loop are present in the avian kidney. Most nephrons are reptilian-like with short or completely absent Henle's loop.

The avian kidneys eliminate nitrogenous compounds, maintain electrolyte balance, normal concentrations of salts and water, regulate acid-base balance, and produce hormones and vitamins (Holz 2020).

6.1.2 Development

The comparison between developing avian and mammalian kidneys shows great similarities in maturation in analogous nephron types (Gambaryan 1992). The urinary system develops embryologically from the intermediolateral mesoderm along three distinct stages. The first primitive system or stage is the pronephros (pronephric kidney) that develops during the early days (1–2) of incubation in the form of tubules lined by simple cuboidal ciliated epithelium with no connection to the future cloaca (early urogenital sinus). This pronephros starts to regress after the development of the mesonephros or mesonephric kidney (day 3), which is fully developed by or at day 7 of incubation (Aryani et al. 2021). The mesonephros consists of extensive tubular structures that connect to the future cloaca. The remnant of the mesonephros gives rise to the reptilian-like nephrons in the definitive kidney cortex. This type of nephron is characterized by short tubules and no Henle's loop. The mesonephros appears morphologically on days 3–4, functions from days 5 to 11, and degenerates about day 15 when it is replaced by the metanephros or metanephric kidney (Bolin and Burggren 2013). The metanephros is characterized by the limited number of long nephrons and Henle's loops to concentrate urine that develops in the medulla of the fully developed chicken kidney.

These future kidneys will induce the degeneration of the mesonephric kidneys and connect through the ureters to the cloaca by developing tubules. These collecting tubules stimulate the nephrogenic mesoderm to start the formation of the glomeruli, (nephrons) and Bowman's capsule. Each nephron connects to the urinary pole of the renal corpuscle.

6.2 Kidney

6.2.1 Macroscopic and Topographic Anatomy

The kidneys are large, elongated, flattened, and dark in color under normal physiological conditions. The kidneys lay on the bony fossa of the synsacrum. The ventral surface

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of the kidney may exhibit elevated regions that should not be classified as distinct gross lobules. This is due to the projection of certain lobules above the surface, with their efferent veins located along the edges.

The avian kidney is composed of a continuous system of ducts. In mammals, the collecting ducts end in the renal papilla, urine is collected in the pelvis and then drains into the ureter. However, in birds, as in reptiles, there is no renal pelvis, and the collecting ducts continue directly into the ureter.

The chicken kidney can be classified as partially lobated and multi-pyramidal. Its cranial border reaches the caudal portion of the lung, while the caudal border reaches the end of the synsacrum (Getty 1975, p. 1919). Left and right kidneys are symmetrical in topographical position.

The chicken kidney is externally divided into three incomplete lobes (cranial, middle, and caudal). These divisions are due to the passage of the external iliac and the sciatic blood vessels. The external iliac blood vessels separate the cranial lobe from the middle lobe, while the sciatic or ischiatic vessels pass through the kidney parenchyma, separating the middle lobe from the caudal lobe (Figure 6.1). The smaller divisions of each lobe into lobules delineating the polyhedral histological lobules are difficult to observe macroscopically. Internally, these lobules are separated from each other by connective tissue and by the interlobular veins, branches of the renal portal veins (Hodges 1974, pp. 489–524) (Figure 6.2). The ventral crests of the lumbar vertebrae and the synsacrum (pelvic bone) separate left and right kidneys.

Medially, the right kidney relates to the caudal vena cava while the left kidney relates to the abdominal aorta and oviduct. Cranially, the left kidney relates to the ovary and adrenal gland. Both kidneys relate cranially to the corresponding testes in the male chicken.

6.2.1.1 Kidney Lobation

Avian kidneys are partially divided into three lobes. Each lobe of the avian kidney can be identified by a secondary branch of the ureter. Each secondary branch drains one single lobe, somehow comparable to the reniculate type of kidney found in pinnipeds. These reniculates do not fuse with each other in marine mammals but they are built within the kidney parenchyma in birds (Figures 6.1 and 6.3).

6.2.1.2 Kidney Lobulation

In the avian kidney, the wedged tissue located between two terminal branches of the renal portal veins forms one lobule. This lobule is pear-shaped and surrounded by collecting ducts (interlobular ducts). Siller and Hindle

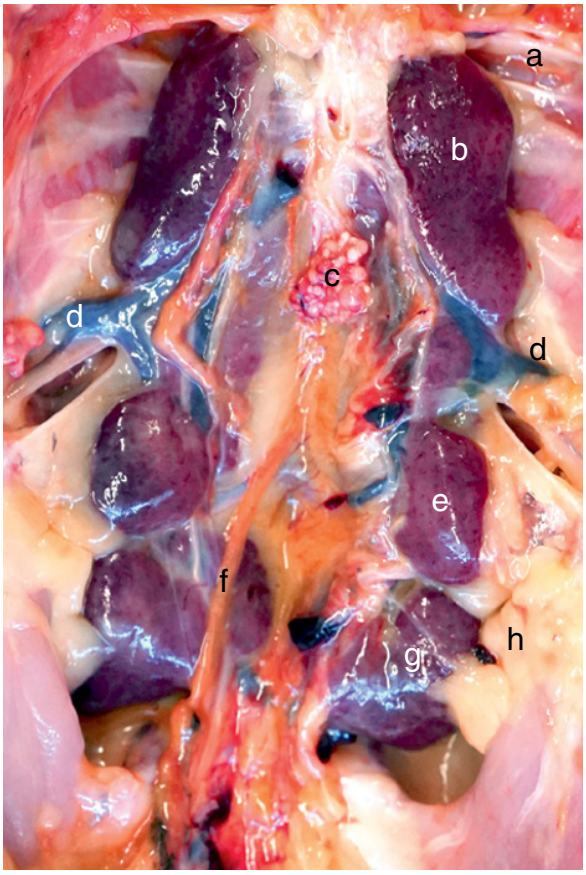

Figure 6.1 Ventro-dorsal view of the celomic cavity/abdominal to pelvic region of a young female chicken showing the two kidneys embedded in the bony synsacrum. (a) Rib, (b) cranial lobe of the kidney, (c) developing ovary, (d) external iliac vein, (e) middle lobe of the kidney, (f) developing left oviduct, (g) caudal lobe of the kidney, and (h) perirenal fat.

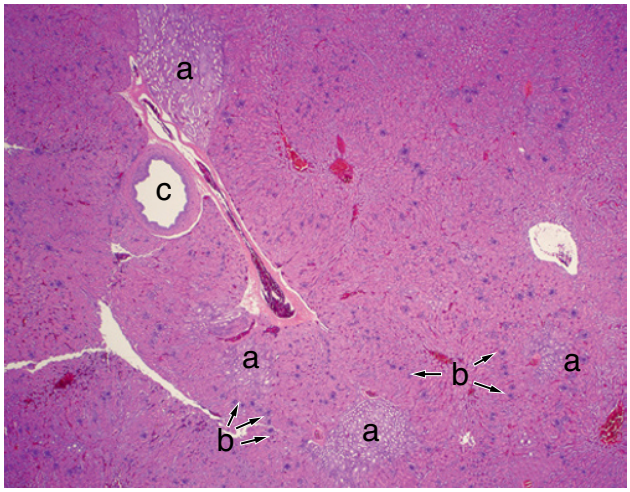

Figure 6.2 A cross section of several lobules within the chicken kidney, (a) several medullary cones, considerable number of glomeruli around each cone, (b) interlobular artery, and (c) intra-renal ureter. H&E stain.

(1969) describe a one-to-one relationship between cortical and medullary lobules in fowl's kidneys. However, other researchers indicated that the typical medullary lobule drains several, usually 3–5, rarely 2, cortical lobules (Johnson and Mugaas 1970).

6.2.2 Histology

6.2.2.1 Kidney Capsule

The renal capsule envelopes the kidney and provides support to the parenchyma. It is composed of a thin layer of fibrous connective tissue rich in collagen fibers and surrounded by a layer of adipose tissue (perirenal fat) that serves as a secondary capsule by providing additional support (Figure 6.4). The amount of this adipose tissue varies in thickness depending on the nutritional status of the bird. The dorsal layer of the kidney connective tissue capsule blends with the periosteum outer layer of the synsacrum, while the ventral layer of connective tissue continues with the sub-serosal connective tissue under the thin parietal epithelium. The parietal layer of the peritoneum covers the kidney's ventral surface, while the dorsal surface is covered by connective tissue, so adventitia instead of serosa may be used to describe the kidney's dorsal outer histological tunic.

6.2.2.2 Cortex

Although histologically the kidney has two distinctive regions, the thicker cortex and the thinner medulla, there is not a clear demarcation between these two regions (Figures 6.3–6.5) (Hodges 1974, p. 490; Dyce et al. 2000, pp. 802–804).

The nephrons make most of the kidney parenchyma. The kidney's stroma comprises the peritubular (interstitial) cells, connective tissue, and blood vessels. According to King and McLelland (1979, p. 207), the cortex is divided into an inner zone enclosed by an outer zone. The proximal tubules occupy the outer zone while inner zone is distal to it and surrounded by the intralobular veins (Siller 1971).

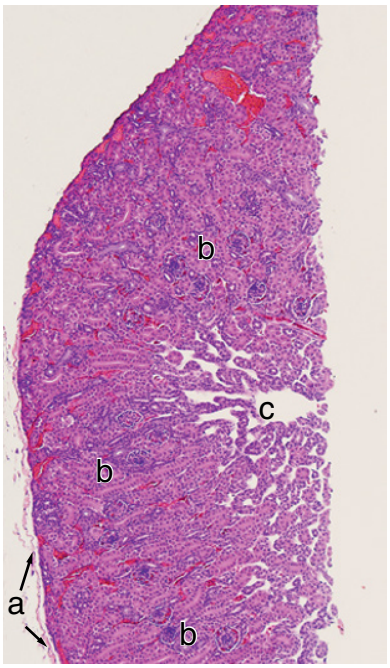

Figure 6.4 Half a lobule of a chicken kidney showing (a) thin connective tissue capsule arrows, (b) cortical tissue with number of glomeruli, and (c) medullary cone of the lobule. H&E stain.

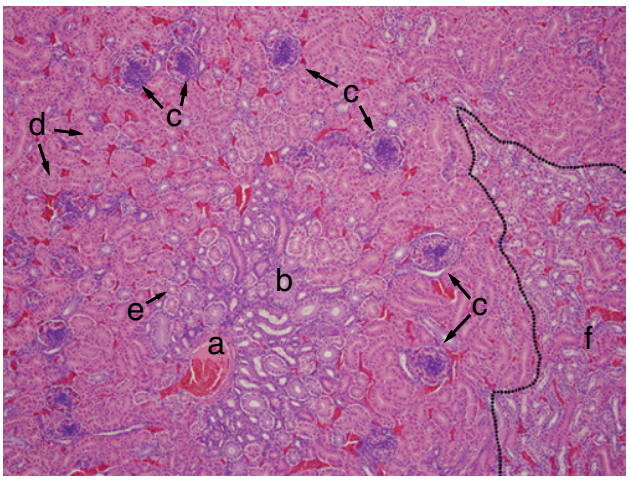

Figure 6.3 A section in one lobule with its (a) blood vessel, (b) within the medullary cone surrounded by (c) glomeruli, (d) thick proximal, and (e) thinner distal tubules. Adjacent medullary cone is labeled (f). H&E stain.

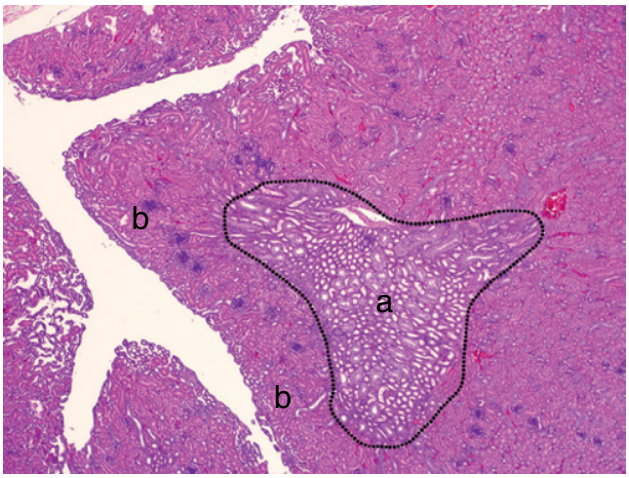

Figure 6.5 A section from chicken kidney showing the (a) medullary cone of one renal lobule which consists of collecting ducts, vasa rectae (not clear at this magnification) and (b) outer cortical tissue surrounds the medullary cone with hypercellular glomeruli-stained purple in color. H&E stain.

6.2.2.3 Medulla

The medulla is a cone-shaped structure surrounded by a connective tissue layer which is continuous with the connective tissue of the ureter. It is thinner than the cortex and it has several cones representing the tip of the pear-shaped lobules. Each cone is made of the medullary collecting ducts, Henle's loop, and the vasa recta (Deepa et al. 2021). The base of the cone faces the cortex while the cone apex communicates with the beginning of the ureter (Figure 6.6). The terminal ducts are the primary ducts which open directly into the ureter. The secondary and tertiary ducts open into the primary collecting ducts. The ureter starts as several collecting ducts that fuse together where the number of vasa rectae decreases.

6.2.2.4 The Nephron

The functional unit of the kidney is the nephron. It consists of the renal corpuscle (glomerulus is also widely used but is a misnomer), the Bowman's capsule, the proximal and distal convoluted tubules, and, in the case of the mammalian-like nephrons, thin and thick tubules.

The type of kidney nephron is contingent upon the complexity of physiological activities, including the kind of metabolism triggered by accelerated food digestion, which in turn, necessitates quicker removal of waste products (Pervenetskaya and Fomenko 2018).

The total number of nephrons has been estimated to be, on average, less than a half million in each kidney (Hodges 1974, p. 504).

Most of the nephrons are found in the renal cortex. The "stalk" of the kidney is referred to as the medullary cone, equivalent to the mammalian renal medulla, and has most of the mammalian-like nephrons and the collecting ducts. The cortical nephrons are the most abundant and resemble the reptilian type with small glomeruli. Reptilian type is limited to the outer cortex, and they are mesonephric in origin. These nephrons are short and without a clear loop of Henle. The long nephrons (mammalian type) have a long loop of Henle located within the medulla and are metanephric in origin. Intermediate type has been described to be in juxtamedullary position with intermediate length nephrons which extend for a short distance into the medulla. Because of the gradation of changes of shape of the nephron between reptilian and mammalian nephrons, Boykin and Braun (1993) suggested to use loopless, transitional, and looped nephrons instead of reptilian, intermediate, and mammalian.

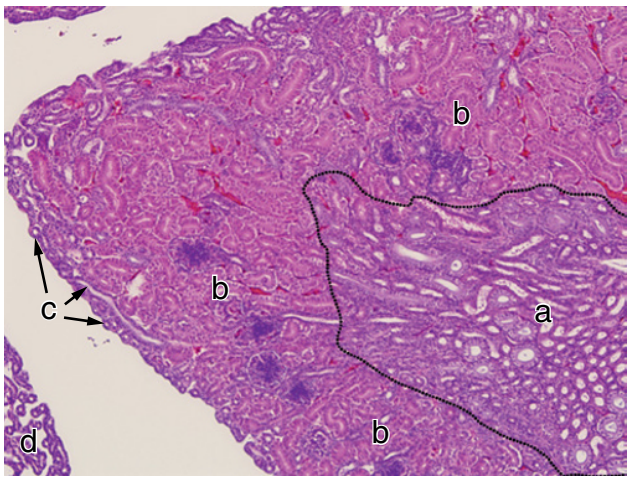

Figure 6.6 A cross section of one renal lobule of the chicken showing the (a) medullary cone surrounded by (b) glomeruli and renal tubules, notice (c) the opening of the ducts at the periphery, (d) a small portion of adjacent lobule appeared on the lower left side of the image. H&E stain.

The medullary nephrons are like the mammalian type. They have large juxtamedullary in position. Hodges (1974, p. 503) and Getty (1975, p. 1923) mention that the chicken has a third type of nephrons, which are intermediate between the reptilian and the mammalian types, although they are infrequently present. Insufficient information regarding this particular nephron type hinders the authors from providing further elaboration on it.

6.2.2.4.1 Glomerulus Although the glomerulus is a vascular structure and not an intrinsic part of the nephron, it maintains a close relationship with the nephron, hence will be described in this section. In the chicken, the glomerulus is a tuft of blood capillaries arranged around the centrally located mesangial cells. The mesangial cells are separated from the endothelial cells of the blood capillaries by a well-developed basal lamina.

The afferent arteriole of the mammalian type nephron is found centrally located within the mesangial cells' matrix but in the reptilian type of nephron, no clear pattern is detected (Morild et al. 1985). There are no differences in the total glomerular number, and their size or volume, between the left and right kidneys and between cranial, middle, and caudal divisions of both kidneys (Wideman et al. 2005).

6.2.2.4.2 Bowman's Capsule Bowman's capsule is a double-layer sleeve that surrounds the renal glomerulus. The outer parietal layer is lined by simple squamous epithelial cells and the inner visceral layer is made of simple cuboidal epithelial cells that follows the glomerular blood capillaries (Figure 6.7). These cells are called podocytes and contribute to the filtration barrier. The podocyte has long pedicels; therefore, the name foot processes are used when describing these cells. Adjacent podocytes leave a small space (slit-diaphragm, filtration

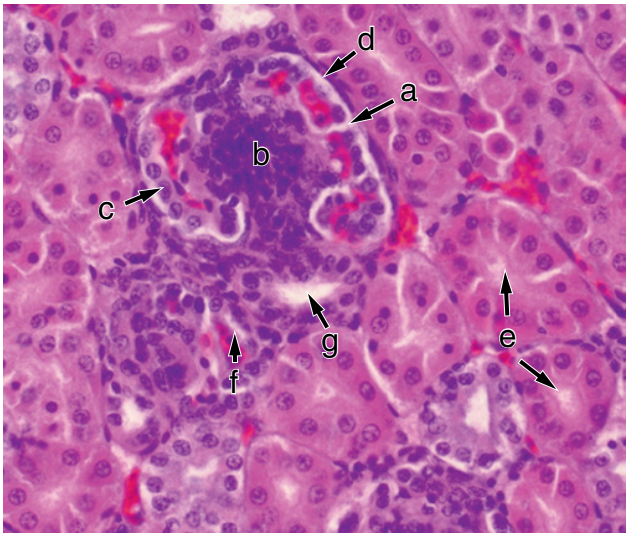

Figure 6.7 Mammalian (outer cortical) type nephron showing juxtaglomerular apparatus, tubules, and cells, (a) urinary space, (b) mesangial cell, (c) visceral layer of Bowman's capsule (podocyte), (d) parietal layer of Bowman's capsule, (e) proximal tubule, (f) afferent arteriole, and (g) distal tubule. H&E stain.

slit, or pore) to allow larger molecules to pass through for filtration to take place. Podocyte and the endothelial cell lining the glomerular blood capillary forms the glomerular filtration barrier.

6.2.2.4.3 Renal Corpuscle The renal corpuscle, also referred to as the Malpighian corpuscle, functions as the blood-filtering unit within the kidney's nephron structure. It comprises a glomerulus, which as mentioned before is a cluster of capillaries lined with endothelial cells, along with a surrounding glomerular capsule commonly referred to as Bowman's capsule.

6.2.2.4.4 Vascular Pole The vascular pole is the apical part of the glomerulus where afferent and efferent arterioles enter and leave the glomerulus, respectively.

6.2.2.4.5 Renal Pole The urinary space is between the parietal and visceral layers of the Bowman's capsule which is where the filtrate drains toward the proximal tubule. The renal pole is the distal part of the Bowman's or urinary space opposite the vascular pole of the glomerulus.

6.2.2.4.6 Filtration Barrier In general, the avian kidney can act as a filter like in mammals. However, Casotti and Braun (1995) mentioned that the chicken has larger sized pores, in both the endothelium and the filtration barrier, than those reported for mammals. These larger sized pores suggest that their glomeruli allow for larger (up to 30% larger than in mammals) macromolecules independently of their charge, to be filtered through the glomerular filtration barrier. A major difference with mammals, which excrete nitrogen in the form of urea and ammonia, is that birds are uricotelic, meaning that they excrete most of their nitrogen residues in the form of uric acid, which occurs as small spheres within the nephron.

The filtration barrier is composed of the adjacent podocyte foot processes connected by tight and gap junctions, and of the endothelial layer of the glomerular blood capillaries with their thick basal laminae. This separation dictates the size of the molecules that pass through it from the blood into the renal space between the two layers of the Bowman's capsule.

Cross and Mercer (1993, pp. 320–321) described the thick basal lamina in mammals to be composed of three layers (lamina rara interna, lamina densa, and lamina rara externa). The major limiting layer for any large size molecule is the lamina densa. An excellent example of large molecules usually trapped in the lamina densa are antigen-antibody complexes. For any disease which mounts a massive immune response to certain antigens, the lamina densa entraps the antigen-antibody complexes which may result in glomerulonephritis. Both, induced and natural glomerulonephritis, has been observed in chickens (Bolton et al. 1988; Wilson et al. 2010), as a consequence of the chicken's thick basal lamina and lamina densa when overwhelmed with immune complexes.

Both mammalian and birds' kidneys have anionic charge barriers to stop certain small molecules passing through despite their small sizes (Casotti and Braun 1996). The difference in filtration of larger molecules in birds may be due to the different arrangement of the anionic barriers or the larger filtration pores as mentioned before (Dantzler 1980).

The negative charge is responsible for preventing anionic proteins from being filtered. It is important to know that both the degree and arrangement of charge sites along the glomerular filtration barrier play a role in this process. This is especially true for birds, considering the large quantity of protein that is present in ureteral urine (Boykin and Braun 1993).

6.2.2.4.7 Proximal Tubule The proximal tubule represents about half the length of the nephron and becomes very convoluted. Medullary nephrons almost double the length of the cortical nephrons with larger luminal tubule diameters. Serial sections of the proximal tubule revealed no evidence of changes in the luminal or the total tubular diameter nor in cell height along its entire length (Wideman et al. 1981). The most obvious change in diameter of the proximal tubule occurs at the juxtamedullary region. The cortical proximal tubule decreases in diameter as it approaches the Henle's loop. Transitions from proximal to distal tubules are abrupt.

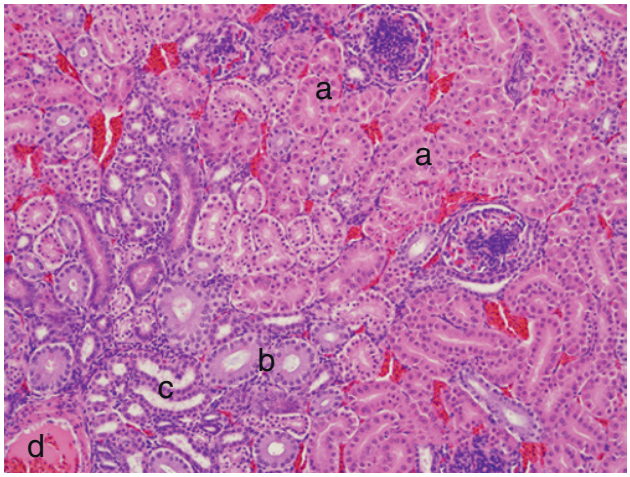

Figure 6.8 Kidney section passing through both the cortex and part of the medullary cone. Notice the large number of (a) proximal tubules with eosinophilic stain cytoplasm in the cortex, while the stain changed into (b) basophilic inside the medullary cone. The epithelium is low leaving larger lumen, (c) the duct system is clear surrounded by blood vessels (congested in certain areas), and (d) intralobular artery is shown at the lower left part of the image. H&E stain.

The proximal tubule is the continuation of the glomerular renal pole of the Bowman's capsule. The constricted neck of the tubule in its origin, as present in mammals, is not evident in the chicken's kidney.

The proximal tubule forms the largest and the predominant tubule in any given cross section of the cortex (Figure 6.8). The complexity of the proximal tubule reflects the complicated function of reabsorption of solutes that pass through the filtration barrier within the glomerulus.

Histologically, the proximal tubule is lined by simple cuboidal to pyramidal (low columnar) epithelium with brush border. The cytoplasm of these cells stains eosinophilic with hematoxylin and eosin while the nuclei are (basophilic). Microvilli projections or brush borders may obscure the lumen when viewed under a light microscope. This tubule also has basal infoldings to increase the surface area and accommodate many mitochondria needed for the high reabsorption activities that occur on this segment of the nephron. However, this feature is not easily observed when the organ is examined under the light microscope without specific mitochondrial stains (e.g. Janus green B, JC-1).

6.2.2.4.8 Distal Tubule The distal tubule starts at the site of contact with the ascending thin limb of Henle's loop and ends at the site of contact with the collecting duct. Like in mammals, it is narrower in diameter and shorter than the proximal tubule. The distal tubule has no brush border and makes a few turns (distal convoluted tubule) before joining the collecting duct. The distal tubule is lined by simple cuboidal epithelium lower in height compared with the proximal tubule; therefore, a large lumen is evident in the distal segment of the tubule (Figure 6.3).

6.2.2.4.9 Mesangium and Mesangial Cells The mesangium is the meshwork of connective tissue between the blood capillaries and the mesangial cells. The mesangial cells are unique to the kidneys, and based on their location within the mesangium can be referred to as intraglomerular or extraglomerular mesangial cells.

The intraglomerular mesangial cells replace the fibrocytes and fibroblasts of the connective tissue. Besides making the cellular component of this tissue, the intraglomerular mesangial cells produce the matrix, rich in fibronectin and laminin, between the tuft of capillaries within the glomerulus.

The intraglomerular mesangial cells interact with the podocytes and contribute to maintaining and regulating blood flow through the glomerular capillaries by their contractile ability which results from the presence of actomyosin filaments which allows for this motility. Mesangial cells have phagocytic capability to remove debris due to cell death within the mesangium. However, the phagocytic activity of these cells is exceptionally low compared to the mammalian counter cells.

Extraglomerular mesangial cells will be discussed under JG apparatus.

6.2.2.5 Juxtaglomerular Apparatus

The JG apparatus is an anatomical structure with the main functions of regulating renal blood flow and glomerular filtration rate. It includes the macula densa of the distal tubule, extraglomerular mesangial cells (Goormaghtigh or Lacis cells) and JG mesangial cells (JG cells or epithelioid cells) of the afferent arteriole. These JG cells are modified smooth muscle cells of the terminal part of the afferent arteriole (Figure 6.9) that contains specific granules having renin (Siller 1971). Although the JG cells are larger in the mammalian type of glomeruli, they are also present in the reptilian type nephrons (Morild et al. 1985).

6.2.2.5.1 Macula Densa Discrepancies in the literature on the presence of macula densa or JG cells plus the identifications of the renin-secreting cells were challenged by several investigators who clearly showed their presence in birds' kidneys of several species, although less developed than those identified in mammalian species. The macula densa (dense spot, MD) is a group of specialized cells located on one side of the distal tubule next to the vascular pole of the renal corpuscle. In chicken, MD cells are not tall columnar like those in mammals and are less dense (Figures 6.7 and 6.9). In contrast to the typical MD in

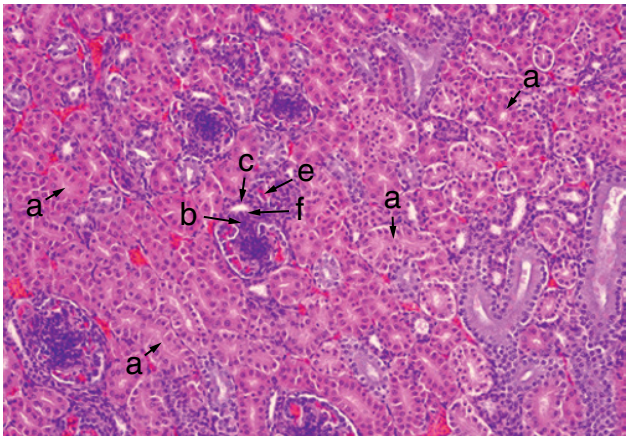

Figure 6.9 Mammalian (cortical) type nephron in the chicken kidney showing the juxtaglomerular apparatus, (a) thick segment of the proximal tubule adjacent to a glomerulus, (b) extraglomerular mesangial cells, (c) distal tubule, (d) afferent arteriole, and (e) macula densa. H& E stain.

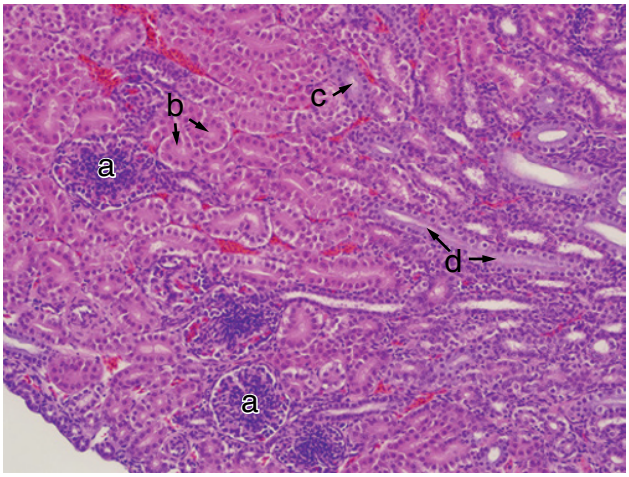

Figure 6.10 Medulla of the chicken kidney showing the mammalian type nephron (long). Notice (a) hypercellular glomerulus, (b) thick proximal tubule, eosinophilic in color, (c) proximal tubule basophilic stained cytoplasm approaching the medulla, and (d) collecting duct lined with simple columnar epithelium and clearly separated cells from each other. H&E stain.

mammals, the cells are neither elongated nor tightly packed (Ogawa and Sokabe 1971). They can be described as high cuboidal cells with less density of nuclei compared to the mammalian MD cells. The internuclear distance was less than those of other distal tubule cells and the height of the cells are not the same. They are densely stained compared to other cells of the distal tubule (Christensen et al. 1982).

6.2.2.5.2 Extraglomerular Mesangial Cells The extraglomerular cells, also called Goormaghtigh or Lacis cells, are present in the angle between the hilar arterioles and the glomerulus. The branching pattern of the basement membrane of the MD is extensive, surrounding the extraglomerular mesangial cells. The basement membrane ends in the walls of both hilar arterioles and in the glomerular capillaries. Thus, all extraglomerular mesangial and glomerular structures are linked together by the branches of the basement membrane. The afferent and efferent arterioles are often found ramifying in the mesangial cell mass. The presence of sympathetic innervation is concluded through the presence of granular epithelioid cells and adrenergic nerves within the glomerulus (Siller 1971).

6.2.2.5.3 Juxtaglomerular Cells JG cells and peripolar cells contributing to distinct JG apparatus were noticed next to the MD and the extraglomerular mesangial cells (Deepa et al. 2020).

6.2.2.6 Collecting Ducts

The most distal component of the nephron tubular system connects to the collecting ducts. Multiple collecting ducts contribute to the formation of the intra-renal ureter. Their main function is to absorb water and sodium ions (Figures 6.2 and 6.10). The collecting duct is lined with low cuboidal epithelium with a clear wide lumen. These cells secrete mucus that helps to keep the duct open and prevent the precipitation of uric acid. The collecting duct cells show similarity with the mammalian tubules as they both have clear lines between adjacent cells. These lines are due to the double straight plasmalemmas (cell membranes) of adjacent cells (minimum modifications and infoldings) which result in intense staining of these structures. This feature enables the viewer to differentiate it from distal tubule under light microscope. The distal tubules have a number of infoldings within the lateral plasmalemmas to allow for larger surface area for exchange compared to the collecting ducts, thus difficult to observe the intercellular separation within the distal tubule.

6.3 Ureter

There are two extra-renal ureters, one in each kidney. Chickens lack renal pelvis. Each ureter has an intra-renal and an extra-renal part (Nickel et al. 1977, pp. 70–72). The ureter starts at the cranial lobe of the kidney and runs caudally on the ventromedial border to collect urine from the collecting ducts of all three lobes. Several collecting ducts will converge or join to form the ureter. In chickens, the ureter exhibits resistance to the flow of urine, potentially impacting the urinary flow originating from the nephrons. Both ureters parallel the ductus deferens in male and the oviduct on the left side of the immature female. In fully

developed adult chicken, the relationship is different because of the complexity of the functional female oviduct. The extra-renal part of the ureter runs on the dorsal aspect of the abdominal cavity above the parietal peritoneum, thus is retroperitoneal until it opens into the urodeum compartment of the cloaca.

The ureters end at the urodeum with a strong sphincter on both the ureteric and the cloacal sides (Gumus et al. 2004). It is also obliquely open into the urodeum with the presence of mucosal fold at the site of opening (King and McLelland 1979, p. 183). The ureter is supplied by branches from all three renal arteries (cranial, middle, and caudal).

Histologically, the ureter has four distinct tunics (mucosa, submucosa, muscularis, and adventitia). The mucosa consists of the epithelium and the lamina propria. Lamina muscularis mucosa is absent in the ureter. The epithelium is pseudo-stratified columnar in the cranial and middle parts of the ureter changing into transitional epithelium in the most distal part where it meets the urodeum of the cloaca (Islam et al. 2001). The entire epithelium is rich with mucopolysaccharides as demonstrated with Periodic Acid Schiff (PAS) stain for carbohydrates detection. The basal cells close to the basement membrane have no reaction to PAS stain. Basal cells are highly regenerative cells that replace the dead columnar epithelial cells. Lamina propria is characterized by loose connective tissue rich in blood and lymphatic vessels and nerves. No glandular tissue was detected in the lamina propria, but mucus was described in the proximal part of the ureter to prevent clogging of the secretory ducts as in the collecting tubules (McNabb 1974). The tunica submucosa has no glands and is composed of irregular connective tissue rich in blood vessels, lymphatic, and nerves. The tunica muscularis is made of longitudinal inner and outer layers with a circular middle layer in between; thus, the ureter is considered to have peristalsis capability. Tunica adventitia surrounds the ureter where blood vessels and innervation access the organ (Figure 6.11).

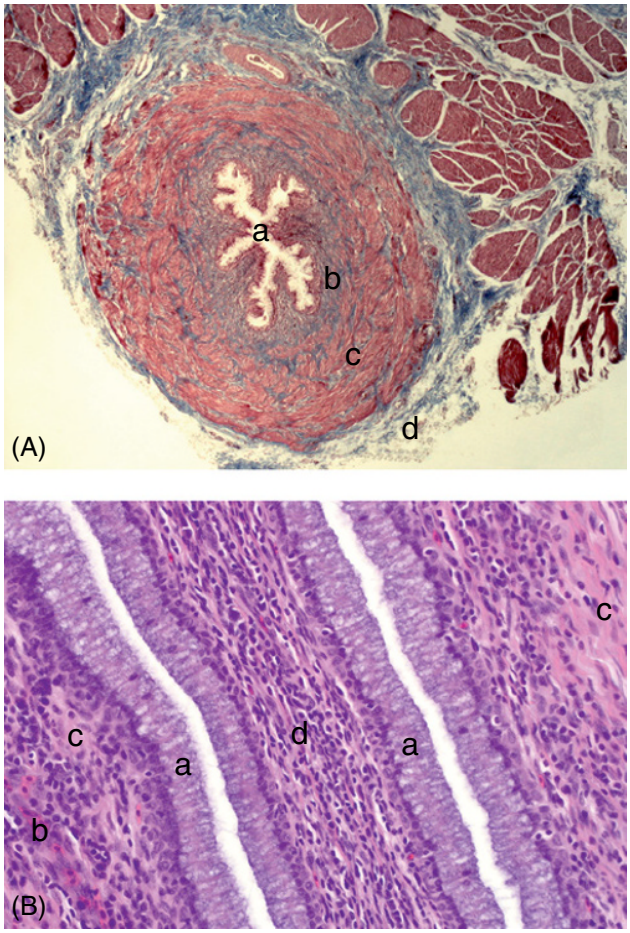

Figure 6.11 (A) Chicken ureter cross section with (a) star-shaped lumen, (b) propria submucosa, (c) thick tunica muscularis, and (d) tunica adventitia. Trichrome stain. (B) Longitudinal section of left and right ureters before opening into the cloaca. (a) Transitional epithelium, (b) vascularity within, (c) layers of smooth muscles, and (d) hypercellularity. H&E stain.

6.4 Urodeum

The urodeum constitutes the central compartment within the cloaca, serving as the site where the ureters open dorsally. It serves as a reservoir for urine collection before its eventual excretion through the proctodeum to the external environment. This segment of the cloaca also receives, on its ventral part, the termination of the genital system, the ductus deferens in the male and the left oviduct in the female. The urodeum is defined by two distinct folds: the caudal fold is known as the uroproctodeal fold, whereas the cranial fold is referred to as the coprourodeal fold. More details on the cloaca can be found in Chapter 4, Digestive System (4.7).

6.5 Blood Supply

Details in addition to the renal portal system are mentioned within *Chapter* 10, *The Cardiovascular System*.

6.6 Innervation

The kidney is found in the sub-lumbar region close to several organs within the dorsal surface of the abdominal part of the coelomic cavity. This region houses many blood vessels and nerve fibers in addition to several sympathetic

ganglia. Therefore, it is not easy to name one ganglion and nerve plexus that are supplying the kidney. In general, the sympathetic nerves supply the kidney is through the renal ganglia and plexus. Adrenergic fibers are demonstrated within the kidney parenchyma (Bennet and Malmfors 1970), while parasympathetic fibers are not evident.

6.7 Urine

The ureter delivers the urine into the cloaca were retrograde peristalsis into the colorectum and into the two ceca is normal physiological process. During urine passage, there is water and ion, such as sodium and chloride, reabsorption. Urates and feces are mixed in the chicken's depositions. Major nitrogenous excretory products of both mammalian and reptilian glomeruli are the urate secretion in the proximal tubule (Laverty and Dantzler 1983; Dudas et al. 2005). In the mammalian kidney, urate is reabsorbed in the proximal tubule at the basolateral surface where exchange usually takes place. Urate-degrading enzyme is absent in birds yet allantoin, a product of this enzyme, has been reported in birds (Simoyi et al. 2003). Most of the urate excretion is done by the proximal tubule and that is how plasma concentration of urate is controlled in chicken (Gutman and Yu 1972).

Urine concentration capability in mammal and avian was proposed to depend on several factors including the difference in thickness of the medulla relative to the cortex, and the location of the glomeruli in the outer cortex relative to those in the juxtamedullary region (longer loop of Henle extends into the medulla which may result in a better reabsorption). Other factors are also considered, such as the complexity of the peritubular capillaries, the type of endothelium, and the basement membrane. The unique reabsorption capability of the proximal tubules in respect to the presence of active pumps and receptors was studied by Kaskel et al. (1987). During early postnatal life, glomerulotubular balance is made possible by a high permeability of the proximal tubule, which compensates for the low net reabsorptive pressure. As the animal matures and the proximal tubule epithelium becomes tighter, for glomerulotubular balance to be maintained, an increase in the number of intercellular channels and in the active transport of sodium need to be postulated. The urinary system concentrating ability varies inversely with body mass; however, birds can concentrate their urine often at two to three times the osmolality of plasma. Further concentration of urine may occur through the retroperistalsis which is a unique phenomenon to birds (Orosz and Echols 2020). Through this process, the urine will be pushed toward the colorectum and back to the cloaca.

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7

Reproductive System

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7.1 Male Reproductive System

The male reproductive system consists of paired testes, epididymis, ductus deferens, and a phallus (copulatory organ).

7.1.1 Testes

The male has two testes (testicle, orchis) that are situated symmetrically in the dorsal portion of the celomic cavity close to the site of embryonic development. They are between the lungs (cranially), the cranial lobe of the kidneys (caudally) and covered by the cranial, caudal thoracic, and abdominal air sacs (King 1975; Nickel et al. 1977) (Figure 7.1). They are attached to the dorsal wall of the celomic cavity by a short double layer of peritoneum called the mesorchion (mesorchium). The mesorchion is continuous with a single layer of peritoneum covering the testis, and through this invagination of peritoneum, blood vessels and nerves reach the testis. Under the mesorchion (peritoneum), the testis is fully surrounded by a fibrous tunica albuginea that projects several rays of connective tissue toward the center of the organ without forming true septa.

Each testis is bean-shaped, with a white to gray or yellowish color in the adult (Figure 7.1). Their size will depend more on the age than the stage of the reproductive cycle, since domestic birds don't have to adjust to natural climatic conditions compared with wild indigenous breeds (Orlu and Egbunike 2010). It is described that the left testis is often larger than the right although no evidence has been found (Mfoundou et al. 2022). Chicken testes grow substantially from hatching to sexual maturation, when semen is produced and birds copulate (up to 12 weeks of age) (McCartney 1978; Mfoundou et al. 2022).

The testicle is surrounded by a thin fibrous and vascular connective tissue, the capsule or tunica albuginea, of approximately 30–60 μm thickness that sends deep inside the gland numerous septa which form the framework or skeleton of the testes (Figure 7.2). This framework creates spaces occupied by the testicular parenchyma. The parenchyma of the chicken testes is composed of coiled **tortuous/convoluted seminiferous tubules** (tubuli seminiferi convoluti/contorti) and the **interstitial tissue** with steroidogenic (Leydig) cells, fibroblast-like cells (mesenchymal cells) and myoepithelial (peritubular) cells (Kirby 1999; Bakst et al. 2007). The interstitial tissue also contains blood and lymphatic vessels, nerves, thin concentric layers of myoepithelial cells, fibroblasts, and connective tissue fibers covering the basal lamina of the seminiferous tubule (Rothwell and Tingari 1973). A reduced basement membrane separates the interstitial space from the seminiferous epithelium of the seminiferous tubules.

The seminiferous tubules contain the germinal cells and the sustentacular or Sertoli cells (see more detail in Section 7.1.5, Spermatogenesis). These tubules are all intertwined, creating a network comparable to that found in the mammalian testes. The convoluted seminiferous tubules continue as the **straight seminiferous tubules** (tubuli seminiferi recti) that are lined by simple cuboidal epithelium which are believed to be tall Sertoli cells (Hodges 1974, p. 316; Budras and Schmidt 1976). This straight seminiferous tubules open into a sequence of interconnected tubules of various diameters that create a network named the rete testis.

The rete testis is located on the medial aspect of the testis and transports spermatozoa rapidly from seminiferous tubules to the efferent ductules from which sperm are conducted into the epididymis. In five-week-old roosters, the rete testis is completely differentiated and lined by a simple cuboidal epithelial layer of 4–7 μm in height, but sometimes the epithelial high is decreased to appear squamous (Hodges 1974, pp. 316–318). Compared to mammalian species, chicken rete testis has three segments: *intratesticular*, the least developed part in all age groups,

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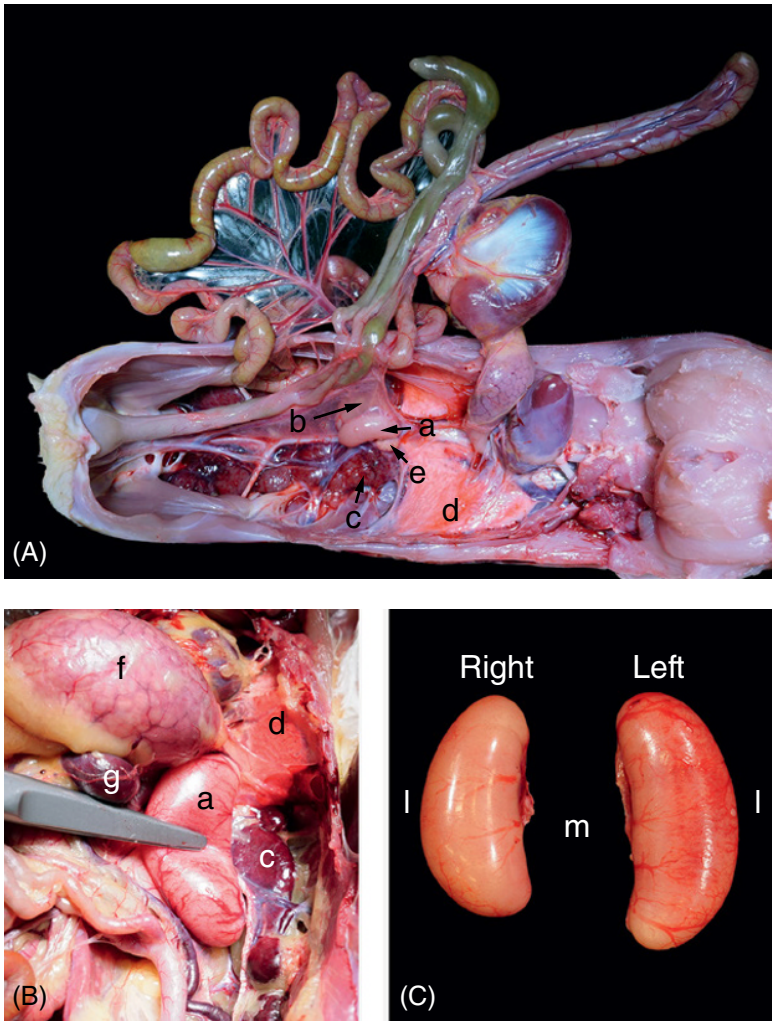

Figure 7.1 Images of the location of the testes inside the abdominal cavity of an immature rooster (A), mature rooster (B) and outside the body (C). (a) Left testis, (b) mesorchion, (c) cranial lobe of the kidney, (d) left lung, (e) left adrenal gland, (f) proventriculus, (g) spleen, (m) medial border of the testes, and (l) lateral border of the testes. Observe the natural landmarks of the testes in situ. The left testis appears larger than the right although there is no literature to support this fact.

despite considerable individual fluctuations, *intracapsular* portion increasingly incorporated into the capsule due to the steady thickening of the testicular capsule lasting until sexual maturity, and the *extratesticular* rete which composes the major part of the rete (Budras and Sauer 1975) (Figure 7.3). Although some fibrous connective tissue is described embedding the rete testis (Hodges 1974, p. 318), the mediastinum testis is absent in birds (Baumel et al. 1993, p. 349). Avian efferent ductules (*ductuli efferentes*) consist of a highly convoluted network of tubules intertwined with the adjacent rete testis and epididymal duct (Janssen et al. 1998). The epithelium of the efferent ductules has numerous folds with a large luminal surface area lined by a simple cuboidal epithelium with ciliated and non-ciliated cells (Aire 1980; Janssen et al. 1998). In sexually mature cocks, this tubular segment has a lumen approximately 140–300 µm in diameter (Budras and Sauer 1975). The efferent ductules of the avian species seem to be the most important component of the excurrent duct system above the rete testis or the epididymis (Clulow and Jones 1988) (Figure 7.3). Their primary functions are reabsorption of approximately 90% of rete testis fluid, concentration and transportation of sperm, protein reabsorption, secretion, and phagocytosis of stagnant sperm (Clulow and Jones 1988; Nakai et al. 1989; Janssen et al. 1998). Comparing with mammals, the male genital tract of avian species is characterized by the presence of extratesticular rete testis, a prominent and physiologically important efferent ductule, and a short and not well-differentiated epididymal duct (Aire 1980; Clulow and Jones 2004).

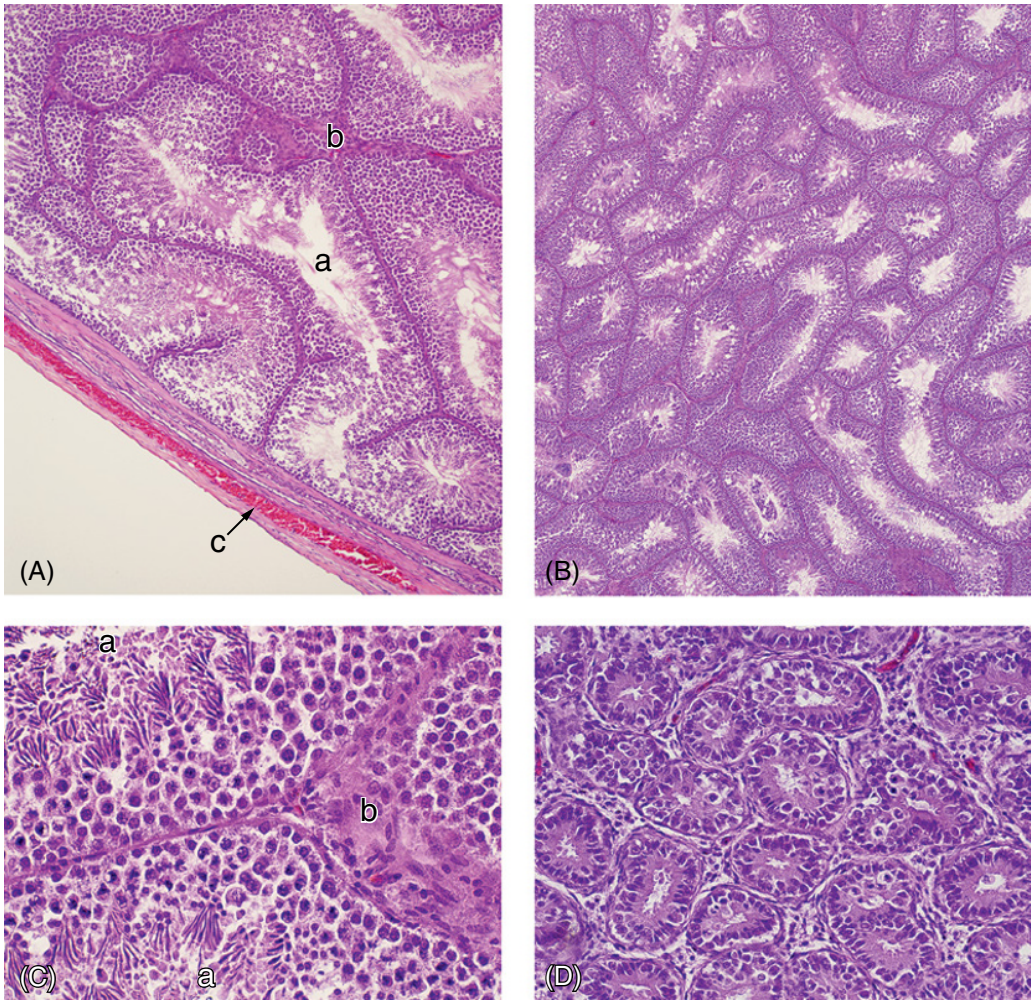

Figure 7.2 Histological sections of mature (A, B, C) and immature (D) chicken testes. H&E stain. (a) Seminiferous tubules, (b) interstitial space, and (c) thin fibrous capsule highly vascularized. Observe the convoluted/tortuous nature of the seminiferous tubules as well as the different cell population inside the tubules. The immature testis (D) shows a single layer of supporting cells (Sertoli) and gonocytes (pre-spermatogonia) inside seminiferous tubules.

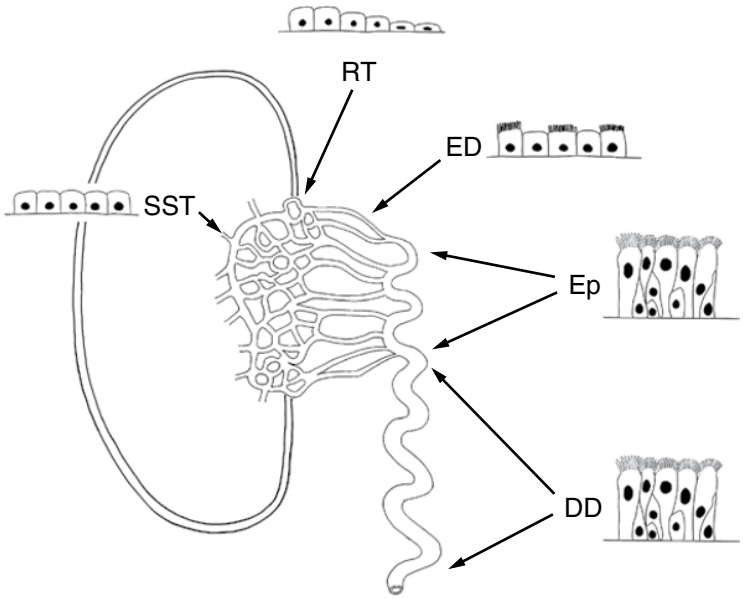

Figure 7.3 Diagrammatic representation of the extratesticular genital duct system in chicken based on the studies of Tingari (1971) and Budras and Sauer (1975). (SST) Straight seminiferous tubules (tubuli seminiferi recti) lined with simple cuboidal epithelium; (RT) rete testis, with an intratesticular, intracapsular and extratesticular portion, lined with simple cuboidal to squamous epithelium; (ED) efferent ductules lined with ciliated simple cuboidal epithelium; (Ep) epididymis lined with ciliated pseudostratified columnar epithelium; (DD) ductus deferens lined with pseudostratified columnar epithelium with cilia or stereocilia.

7.1.2 Epididymis

The avian epididymis is a single duct, shorter and much less convoluted than in mammals. There are no clear demarcations between different segments of the epididymis as the efferent ductules open over all its length (Figure 7.3). It is located on the medial border of the testis. In the sexually mature cock, the lumen of the epididymis, which is usually filled with a mass of closely packed spermatozoa, is considerably dilated (between 190 and 270 μm) in diameter in the initial segments but can reach 400 μm in the caudal segment. This dilation of diameter helps distinguish the epididymitis from the efferent ductules, in addition to the histologically characteristic brush border lining the lumen (Budras and Sauer 1975). The stored sperm is detached from the surface of the cell to the lumen center of the epididymis due to the numerous vesicular protrusions. The epididymis has a circular outline in a cross section. It is composed of an outer connective tissue layer and an inner lining of pseudostratified columnar epithelium about 30 μm in height (Hodges 1974, p. 320) (Figure 7.4). No secretory activity were reported within the epididymis.

It is well known that the mammalian epididymis is an accessory reproductive organ where sperm maturation occurs through changes in lipid and protein composition of the sperm plasma membrane (Jones 1998), acquiring motility and fertilizing capabilities. However, the role of the avian epididymis in sperm maturation is not well described.

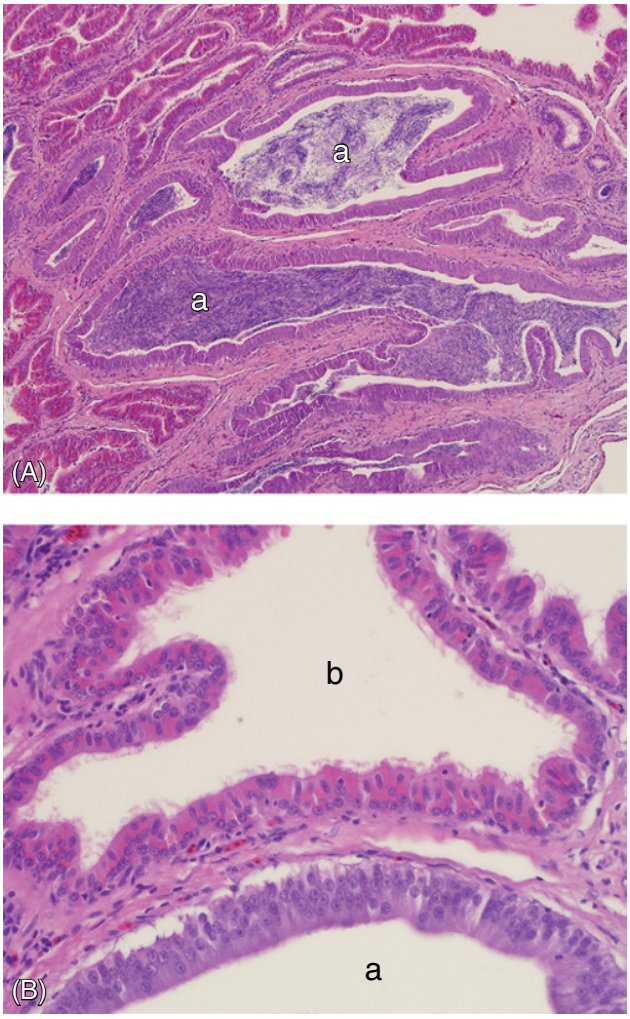

Figure 7.4 Histological sections of epididymis (A and B). H&E stain. (a) Epididymis filled with a mass of closely packed spermatozoa. (b) Efferent ductules with large cavities and an epithelium that transition from a simple ciliated cuboidal to a pseudostratified ciliated typical of the epididymis.

7.1.3 Deferent Duct (ductus deferens)

The deferent duct (ductus deferens, vas deferens) is the continuation of the epididymis, and it is relatively difficult to differentiate from each other in its initial segment. The epididymis is confined within the connective tissue of the juxtatesticular region, but the ductus deferens is free (Tingari 1971). The deferent duct runs caudally and retroperitoneally toward the cloaca, close to the synsacrum and in contact with the ventromedial surface of the kidney along with the ureter (Figure 7.5). The deferent duct is highly tortuous with a small lumen and, close to the opening into the cloaca, expands and straightens to terminate into the dorsal wall of the cloaca in a small, elevated papilla inside the urodeum close to the opening of the ureter.

Histologically, the ductus deferens is externally surrounded by a dense layer of connective tissue under the peritoneum. Below this layer, there is a thick layer of smooth muscle not clearly differentiated into circular and longitudinal layers. The mucosa is lined by a pseudostratified columnar epithelium with stereocilia and without glands, similar to the epididymal epithelium (Hodges 1974). There are no accessory sex glands described in chicken. The luminal diameter of the ductus deferens transitions from 400 μm in the initial segment to 550 μm in the middle and more than 900 μm toward the caudal portion close to the cloaca (Tingari 1971) (Figure 7.6).

7.1.4 Phallus

In birds, the phallus is the male copulatory organ, and it is a non-protrusible intromittent structure. The phallus can be found on the ventral lip of the vent and has a spiral phallic sulcus or groove inside which ejaculate passes. It consists of a median white phallic body (few millimeters in diameter) and two lateral phallic bodies (2–4 mm) surrounded by two phallic folds. However, domestic fowl

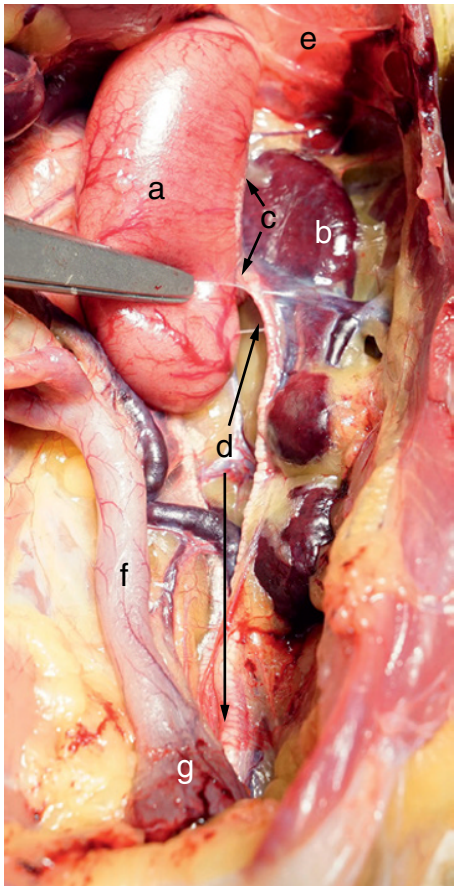

Figure 7.5 Location of the epididymis and deferent duct inside the celomic cavity. (a) Left testis, (b) cranial lobe of the left kidney, (c) epididymis, (d) deferent duct, (e) caudal border of the left lung, (f) colorectum, and (g) cloaca (coprodeum).

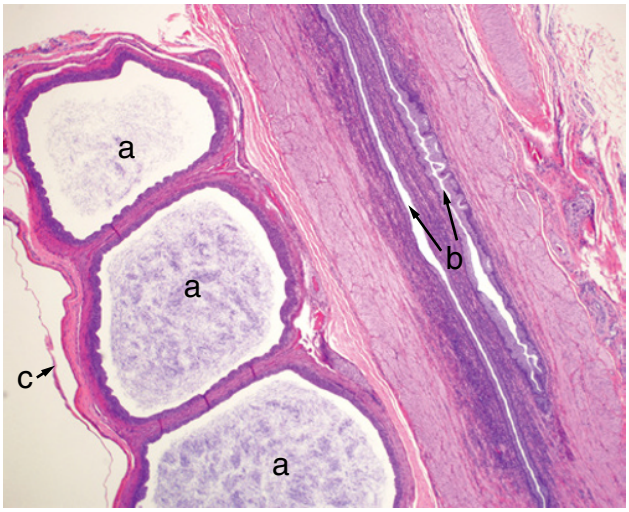

Figure 7.6 Histological sections of deferent duct close to its termination to the cloaca (urodeum). H&E stain. (a) Deferent duct with a large lumen and sperm inside. Its tortuous arrangement allows to show —two to three cross sections. (b) Right and left ureters traveling close to each other close to their termination to the cloaca (urodeum). (c) Thin connective tissue capsule.

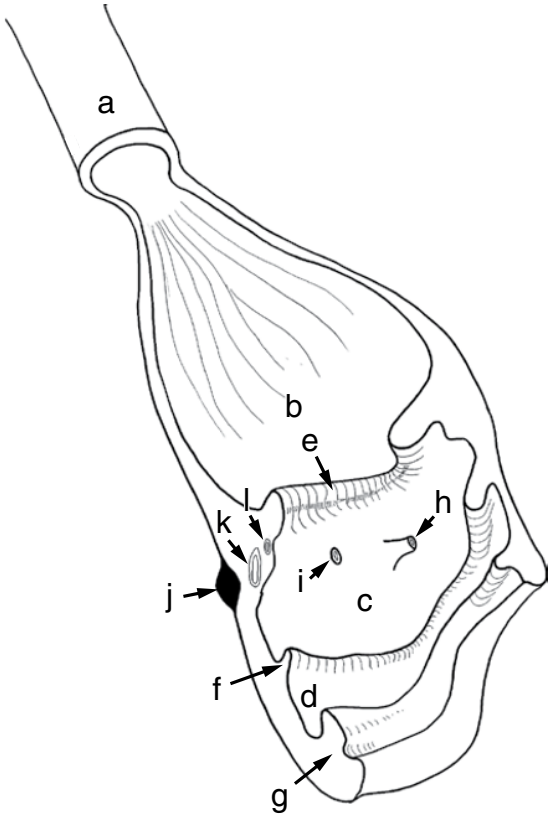

Figure 7.7 Diagram redrawn and based on King (1975) and (1993) of the ventral quarter of the male cloaca and vent of a rooster. (a) Rectum, (b) coprodeum, (c) urodeum, (d) proctodeum, (e) coprourodeal fold, (f) uroproctodeal fold, (g) ventral lip of the vent, (h) papilla of the deferent duct, (i) orifice of the ureter, (j) corpus vasculare phalli, (k) ductus deferens, and (l) ureter.

does not possess a true phallus as described. Instead, it has a copulatory organ within the cloaca where each deferent duct connects with a conical protrusion (papilla-like erectile ejaculatory duct) near the ureteral openings (Hodges 1974, p. 326; King 1975). These ejaculatory ducts are approximately 2.5-mm long but can enlarge and erect prior copulation. There is a plexus of arteries and veins (corpus vasculare phalli) at the base of the papilla, believed to serve as the erectile tissue during ejaculation (Fujihara 1992), and the tissue in the vicinity of the papilla is responsible for the secretion of a lymph-like fluid that mixes with the semen during the ejaculation (Knight et al. 1969; Fujihara 1992) (Figure 7.7).

7.1.5 Spermatogenesis

Male fertility requires that the testis produces large number of normal spermatozoa through a complex process known as spermatogenesis. Spermatogenesis is defined as the complete process of sperm cell production from the

male initial germinal epithelium. On the other hand, and not to be confused, spermiogenesis is the final differentiation and maturation process of the spermatids into sperm cells. During the process of spermatogenesis, the diploid spermatogonia differentiates into the final haploid spermatozoa. This is a complicated process that includes spermatocytogenesis which entails mitotic proliferation of spermatogonia, meiotic division of primary spermatocyte into round spermatid, and spermiogenesis that includes morphological differentiation of round spermatid into the final shape spermatozoa (Thurston and Korn 2000). Although spermatogenesis is extensively studied in mammals, there are only limited reports detailing spermatogenesis in Aves (Asano and Tajima 2017). The cytological relationships of the cells comprising the seminiferous epithelium of several avian species have been reported in Japanese quail (Jones and Lin 1993), turkey (Bakst et al. 2007), and chicken (Thurston and Korn 2000).

Prior to sexual maturation in the chicken, interstitial tissue (steroidogenic and mesenchymal cells) is extensive, while the seminiferous tubules comprise a single layer of supporting cells (Sertoli) and gonocytes (prespermatogonia) along the basal lamina. After one month of age, seminiferous tubules complete their formation and allow spermatogonia to differentiate into primary spermatocytes and start displaying more layers of cells, displaying a great increase in size between two-month and three-month-old (Mfoundou et al. 2022). The left and right testes grow in a similar way and their increase in length and diameter is related to the spermatogenic activity happening within the seminiferous tubules (Mfoundou et al. 2022). The walls of these tubules are composed of connective tissue arranged as a tunica propria and a basement membrane where the germinal (spermatogonia) and sustentacular (Sertoli) cells are attached to. Seminiferous tubules can reach 300 μm in diameter during sexual maturity. The testes exhibit all spermatogenic cells with colonies of spermatozoa noted on the tubular epithelium and within their lumen, coinciding with sexual maturity.

From the periphery of the seminiferous tubule epithelium to the lumen more mature and differentiated germ cells can be observed in the following order: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and maturing spermatozoa. The Sertoli cells are among this sequence of germ cells providing support, protection, and nutrition to them (Figure 7.8). Although the cytoplasm of the Sertoli cells cannot be recognized easily, it has been shown that they form multiple ramifications around the germinal cells creating close contact with them. The resting Sertoli cell is found near the basal compartment of the seminiferous tubule, with a large basal pale nucleus, and with abundant mitochondria and Golgi

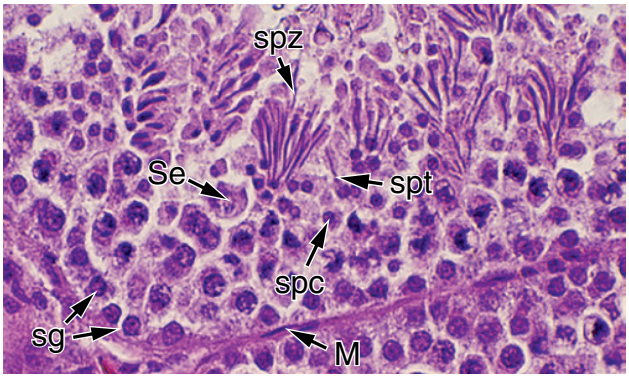

Figure 7.8 Higher magnification of seminiferous tubules inside the testis. H&E stain. (M) myoepithelial peritubular cell, (sg) spermatogonia at the base of the tubules at the basal compartment, (Se) Sertoli cells, (spc) spermatocyte, (spt) spermatid, and (spz) spermatozoa.

apparatus toward the apical portion of the cell. The Sertoli cells are adhered to each other by tight junctions, macula adherens, and gap junctions (Cheng and Mruk 2015) contributing to the blood-testis barrier (BTB) or Sertoli-cell barrier. This barrier divides the seminiferous tubule into a basal compartment (outer side of the tubule, toward the blood and lymph) and an adluminal compartment (inner side of the tubule, isolated from blood and lymph). The concept of BTB was discovered in the nineteenth century, when dyes injected into the circulatory system of rodents showed that the brain and the seminiferous tubules were not stained. The benefits of the presence of the BTB are: (i) to protect the final stages of the germ cells from blood-borne noxious agents (toxic or microorganisms), (ii) to provide an immunoprivileged microenvironment for the completion of meiosis by avoiding the generation of an autoimmune response due to the expression of antigenic molecules during the process of germ cell maturation after puberty, and (iii) to establish an osmotic gradient that facilitates movement of fluid from plasma into the seminiferous tubular lumen due to the different composition (Mruk and Cheng 2015).

7.1.6 Mature Spermatozoa

The spermatozoon is a very specialized and differentiated cell evolved to achieve the goal of fertilization. One cubic millimeter of the ejaculated fluid called semen produced by the male contains on average three to five million sperm. Although the morphology between mammalian and avian spermatozoa is significantly different, they share the classical division of these main parts: head, neck, and tail (Figure 7.9). Avian spermatozoa is smaller than the average mammalian sperm. The typical morphology of chicken spermatozoa is

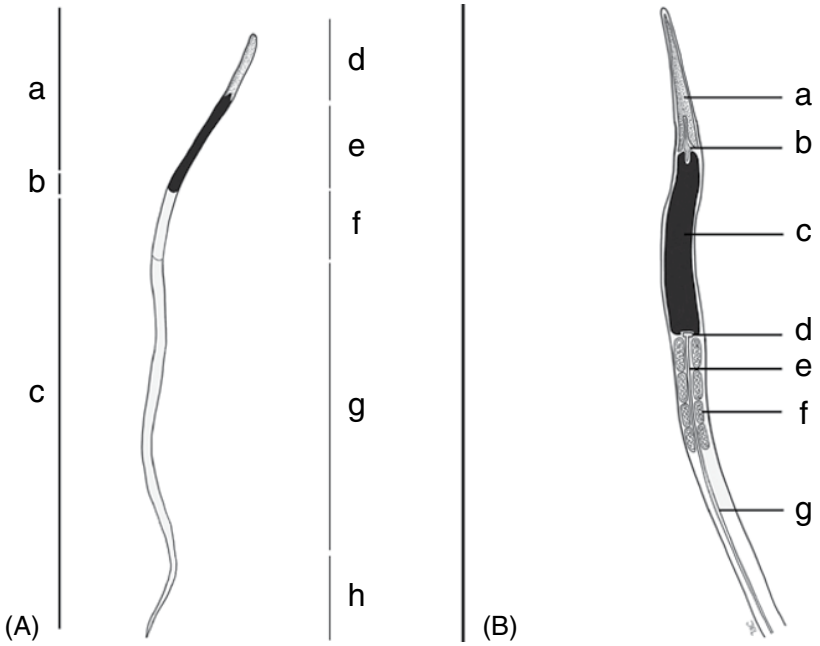

Figure 7.9 Diagram of a rooster sperm morphological features. (A) Entire spermatozoa. (a) Sperm head, (b) neck, (c) tail (flagellum), (d) acrosomal region, (e) nucleus, (f) midpiece with mitochondria, (g) principal piece, and (h) end piece. (B) Detailed region of the sperm head. (B) Details of the sperm head and midpiece. (a) Acrosome (with internal, external acrosomal membrane, and acrosomal matrix), (b) perforatorium or spine, (c) nucleus (with double nuclear membrane), (d) proximal centriole, (e) distal centriole, (f) mitochondria, and (g) central flagellar microtubules.

vermiform with a maximum width of 0.5–0.7 µm and lengths of 90–105 µm, and an average volume of 9.3 μm^3 (Thurston and Hess 1987; Long 2006; González-Santos et al. 2019).

The rooster sperm head has a filiform morphology (long and narrow, 0.5–0.7 µm in length and 0.3–0.4 µm in width), and it differs from the shape of sperm heads from other birds. The head contains two main cellular compartments, the acrosome and the nucleus. The acrosome is a cap-like structure (a vesicle derivate from the Golgi apparatus) covering the anterior portion of the nucleus and encapsulated by the sperm cell membrane. It has an outer and inner acrosomal membrane and contains proteases and receptors required for sperm interaction with the ova. At its base, the acrosomal cap encircled apical projections of the nucleus. The nucleus consists of condensed DNA, and it is covered by a reduced nuclear envelope. For turkey and chicken spermatozoa, the neck is relatively short and connects the head with the tail. This region is connected to the midpiece and consists of a proximal centriole and its pericentriolar processes oriented perpendicularly to an elongated distal centriole (Thurston and Hess 1987). The distal centriole persists in avian, but not in most mammalian sperm, and serves as the precursor of the central tubules of the flagellum (Maretta 1977), the main constituent organelle of the tail. The sperm tail can be divided into four major segments that share a common innermost structure that is the flagellum but differ in their external substructure. From proximal to distal, the sperm tail starts at the neck region or connecting piece, the midpiece associated to the mitochondria close to the proximal end where there are 25–30 helically arranged mitochondria that produces the energy and the necessary ATP (adenosine triphosphate) for sperm motility, the principal piece, and the end piece. The flagellum is based upon the 9 + 2 arrangement of microtubules within the sperm flagellar axoneme. The 9 + 2 arrangement refers to nine peripherals, symmetrically arranged microtubule doublets connected doublet-to-doublet by dynein arms and to the sheath of central pair of microtubules by radial spokes (Sutovsky and Manandhar 2006, p. 6). However, the typical outer dense fibers are absent in birds (Thurston and Hess 1987).

7.2 Female Reproductive System

The female reproductive system consists of a single ovary and a single oviduct. While the chicken female embryo has two sets of reproductive organs, only the left remains and reaches maturity to produce eggs. The remnant of the right genital organ in the adult hen is called regressed right cystic oviduct normally visible in young birds and rudimentary in adult hen (Figure 7.10).

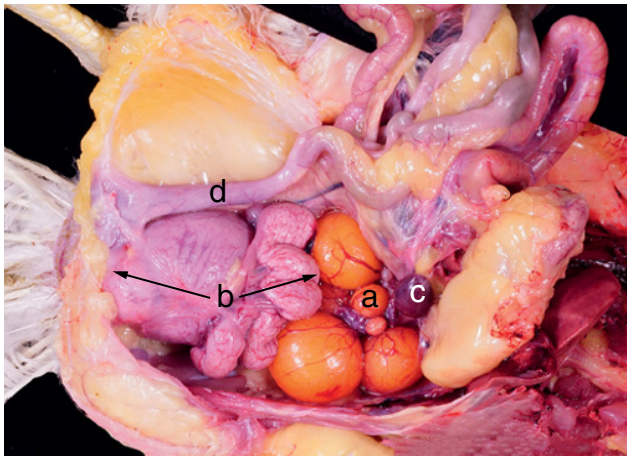

Figure 7.10 Female reproductive tract of a mature hen (in situ). (a) Mature ovary, (b) oviduct, (c) spleen, (d) colorectum.

7.2.1 The Ovary

In the laying hen, the single persistent ovary is located just in front of both kidneys in the dorsal celomic abdominal cavity, like the testes in the male, and it is firmly attached to the wall of the cavity by the mesovarium (pedicle). Adjacent to the ovary in the female, and the testes in the male, just anterior to the cranial lobe of the kidney, lies the adrenal gland.

Since the persistent ovary is the left one, it is slightly situated toward the left of the peritoneal (celomic abdominal) cavity. The ovary is well provided with blood vessels to ensure there is no hindrance to the transport of nutrients to the developing yolk. The ovary is a granular irregularly shaped organ, like a bunch of little grapes, made up of 3500–5000 small whitish-pale spheres of underdeveloped follicles (each containing an ova and yolk) (Figure 7.11). Shortly after hatching, the ovary (inactive) is a small structure of 5×2×1 mm with a triangular shape, yellowish white in color. In contrast, the active ovary is an organ of considerable size, and it contains follicles (ova, yolks) at different stages of development (primary, growing, mature, released, and atretic) that can reach 3–5 cm in diameter. Only one follicle is fully developed at a time, although the successive maturation of follicles can be a few hours apart. There is no corpus luteum described in the chicken ovary at any stage. The mesovarium of the mature ovary is thickened and contains fibrous connective tissue, smooth muscle, blood vessels, and nerves (Hodges 1974, pp. 327–328).

Histologically, the surface of the ovary is covered by a single layer of germinal epithelium described as simple cuboidal to columnar epithelium depending on the region of the ovary and the size of the follicle that cover. Underneath this epithelium, there is a layer of connective tissue called *tunica* albuginea. The functional portion of the ovary consists of an outer cortex (zona parenchymatosa) containing the follicles, and a highly vascular medulla (core or zona vasculosa) composed of connective tissue and blood vessels (Figure 7.12). When a follicle develops, it separates from the medulla, and

Figure 7.11 Ovaries of a hen. (A) Mature and active ovary with follicles at various stages of development (first to ovulate at the top right). (B) Immature ovary (a) inside the peritoneal (celomic) cavity, (b) cranial, (c) middle, and (d) caudal renal lobes (divisions).

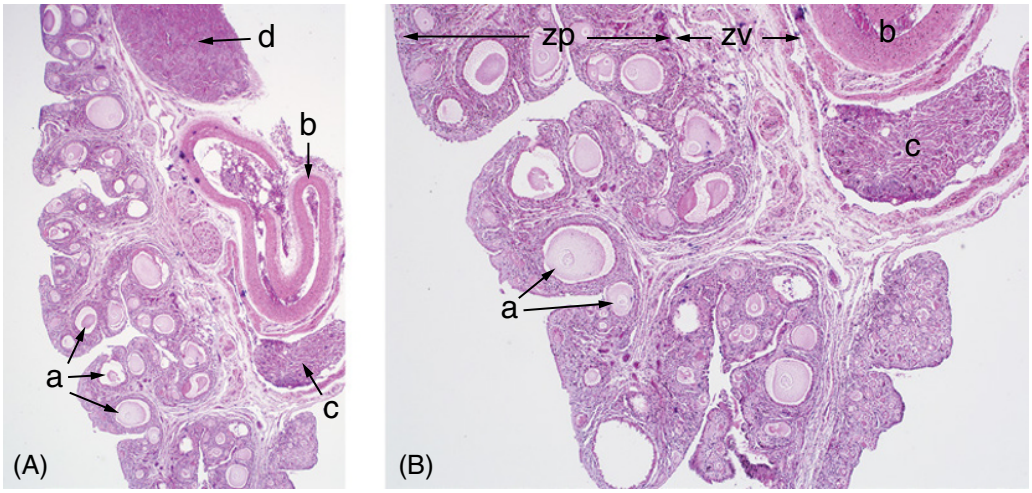

Figure 7.12 Histological section of a hen's ovary (A and B). H&E stain. (a) Developing follicles of different stages, (b) ovarian vessels, (c) autonomic ganglion associated to the ovary, (d) small portion of the left kidney, (zp) zona parenchymatosa (cortex), (zv) zona vasculosa (medulla).

it is only attached by a delicate fibrous pedicle that carries the blood supply. When the yolk inside the follicle is fully formed and mature, it escapes the fibrous capsule through a cleavage called stigma (Figure 7.13). A mature follicle consists of the oocyte, a single layer of columnar follicular epithelium at the early stages and becomes flattened squamous during the growth of the follicle, and the fibrous thecal layer. The thecal layer is very vascular, and the arrangement of the concentric alternating layers of collagen and fibroblasts differentiate into theca externa and theca interna (King 1993, p. 375, in Baumel et al., 1993).

The blood supply of the ovary comes from two to four ovarian arteries arising normally as direct branches off the left branches of the cranial renal artery alone (most common pattern), branches from the aorta alone (less common pattern), or from both, the aorta and the left cranial renal artery (Hodges 1974, p. 328; King 1993, p. 376, Baumel et al. 1993). The innervation of the ovary passing through the ovarian pedicle comes from the ovarian plexus which is connected to the celiac, cranial mesenteric, aortic, and adrenal plexuses. It is described as a bundle of nerve fibers and ganglia shared with the surrounding structures such as the adrenal glands (Hodges 1974, p. 329; King 1993, p. 377, in Baumel et al. 1993).

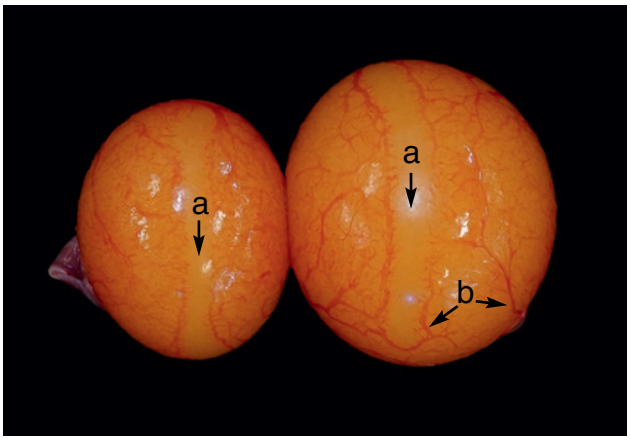

Figure 7.13 Mature follicles (yolk) of different size or developmental stage. (a) Stigma and (b) thecal vessels.

7.2.2 The Oviduct

The left oviduct is a muscular tube that transports the ovum from the ovary to the cloaca (urodeum). It is attached to the body wall by a mesentery (double layer of peritoneum) called the mesotubarium with a dorsal and a ventral portion. The oviduct elongates from 15 cm in the non-laying hen to almost 90 cm in the laying hen. However, the most common size for modern chicken laying breeds is around 60 cm (Gilbert 1979, p. 308). When inactive (non-laying), it is visible as a pale pink, semitranslucent structure ventral to the left kidney, connecting the inactive ovary to the cloaca. When active, it occupies the left half of the abdominal cavity and may protrude into the right. Due to its role in egg production (all but the yolk), it is very glandular and well vascularized.

Histologically, it is constituted by several layers that vary in size and characteristics depending on the specific oviductal segment. From the luminal layer to external, the layers are: tunica mucosa with epithelium and lamina propria, tela submucosa, tunica muscularis with circular (internal) and longitudinal (external) strata, and tunica serosa.

The oviduct is composed of five distinct segments: infundibulum, magnum, isthmus, uterus, and vagina (Figure 7.14).

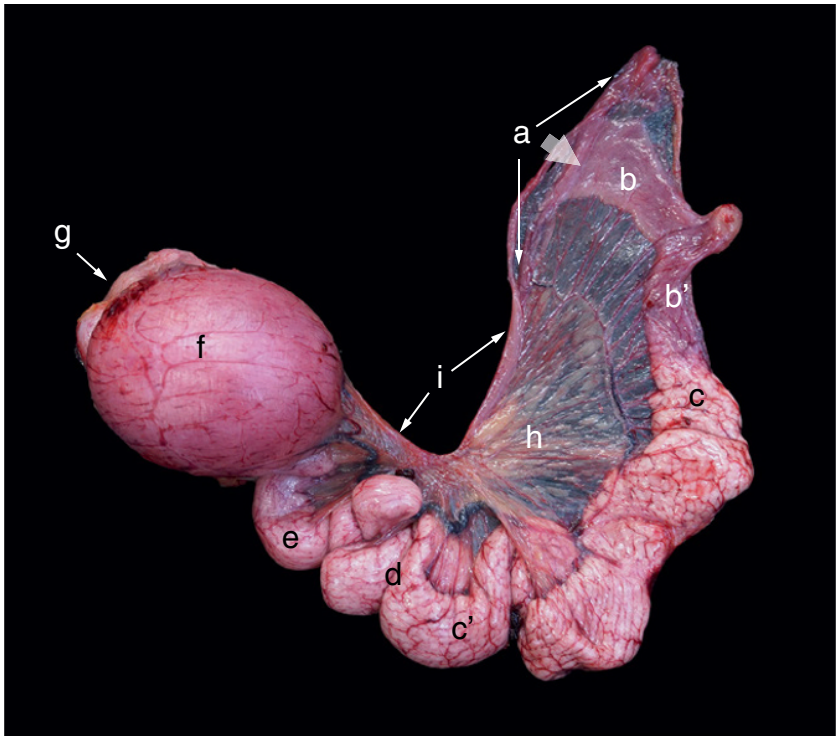

Figure 7.14 Excised hen's oviduct. (a) Entrance of the infundibulum, white wide arrow indicates the infundibular opening, (b-b') infundibulum, (b) funnel portion of the infundibulum, (b') neck of the infundibulum, (c-c') magnum, (d) isthmus, (e) uterus or shell gland, (f) vagina, (g) cloaca, (h) mesotubarium (ventral), and (i) muscular cord.

7.2.2.1 Infundibulum

This segment of the oviduct is thin-walled, funnel-shaped, and the main opening (ostium infundibulare) faces the ovary with the function of collecting the oocyte (ova, yolk). At the periphery of the free border of the infundibulum, the finger-like projections that extend toward the ovary are called fimbriae. The infundibulum length is close to 9 cm in laying hens and is much shorter in non-laying hens. It can be subdivided into the funnel (widest portion) close to the ovary and approximately 8 cm in diameter and the neck. The thin funnel portion converges to the neck, the narrow portion that increases the diameter and thickness of the wall to form the magnum.

The infundibulum is externally covered by peritoneum (mesothelium) overlying connective tissue embedded with smooth muscle bundles scattered and not well delineated to form a circular and longitudinal layer (Figure 7.15). Internally, the infundibulum is lined by ciliated low columnar epithelium with few scattered goblet cells to facilitate the transition of the released yolk toward the second portion of the oviduct. The mucosa is provided with low ridges oriented longitudinally that becomes spiral toward the magnum (Hodges 1974, p. 352) (Figure 7.15).

The yolk remains within the infundibulum for around 15 minutes, and it is here where fertilization normally occurs. The serosal ligaments associated with the infundibulum form an "ovarian pocket" bringing the infundibular ostium into a suitable position to accept the released oocyte (Gilbert 1979, p. 311 in King and McLelland 1979). If the oocyte (yolk) fails to enter the infundibulum, there is a process called "internal laying" in which egg yolks are not captured by the infundibulum and are lost into the body cavity (Greenwood and Blyth 1938). In this situation, a loss yolk could be found in the ovarian pocket where they can remain for several days and be reabsorbed or freed into peritoneal cavity. The internal laying is believed to be due to the lack of synchrony between the maturation of the oviduct and ovary at the age of sexual maturity (Melnychuk et al. 1997).

7.2.2.2 Magnum

The magnum is the longest segment of the oviduct which can reach an average of 32–40 cm and its main function is to participate in the formation of at least 50% of the thin and thick albumen on the developing egg (see Chapter 15, The Egg Anatomy). Although the formation of the chalaza starts at the neck of the infundibulum, the main structure is produced and finished in the magnum.

The diameter of the magnum is greater than the infundibular neck segment and it is due to the increase in thickness of the wall. The magnum wall is thicker as a result of

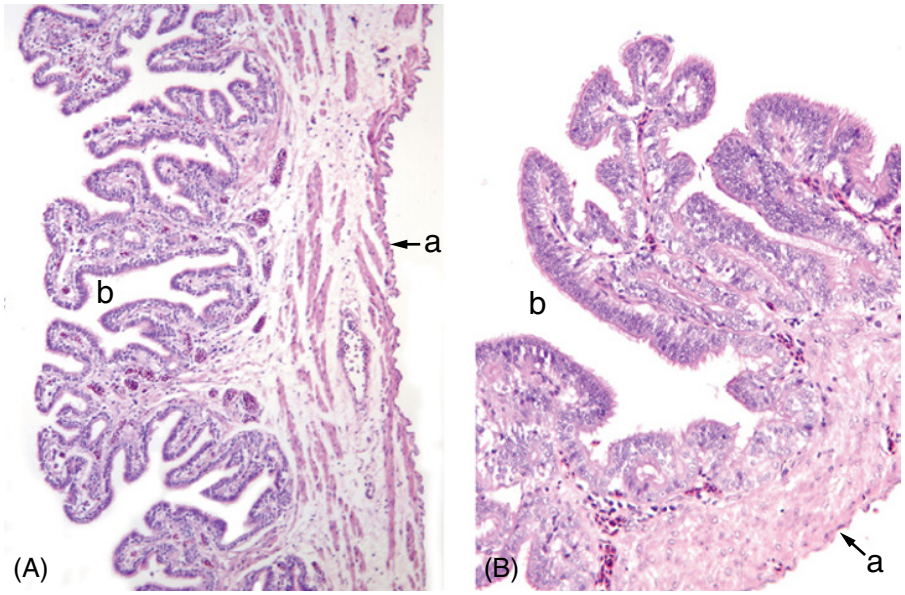

Figure 7.15 Histological section of a hen's infundibulum (A and B). H&E stain. (a) Peritoneum (mesothelium) covering externally the infundibulum and (b) mucosal folds of the infundibulum lined by ciliated low columnar epithelium with few scattered goblet cells.

an increased muscular layer thickness, development of well-developed branched tubular glands in the propria-submucosa, and the increased thickness of the mucosal layer. The mucosa features coarse, tall folds arranged spirally, facilitating the rotation of ova inside the magnum during their descent. The yolk must remain centrally located for the survival of the embryo and the chalazae are these structures that hold the yolk in this central position. The chalazae are cords that extend from the egg poles to the equatorial envelope of the yolk (see Chapter 15, The Egg Anatomy). The yolk turning or rotating as it passes along the magnum causes the twisted effect of the chalazae.

The mucosa of the magnum has the above-mentioned folds (4- to 5-mm tall and 2- to 3-mm thick) (Hodges 1974, p. 361) lined by ciliated simple columnar epithelium with large number of goblet cells (Figure 7.16). The thickness of the epithelium depends on the segment ranging from 10 μm at the proximal (upper) magnum to 25 μm at the distal (lower) magnum indicating a larger secretory capability (mucous region) (Hodges 1974, p. 362). In the mucosa and submucosa (propria-submucosa), there are well-developed branched tubular glands (Figure 7.16). Not all the glands within an area of the magnum are at the same phase of the secretory cycle alternating between secretory and resting

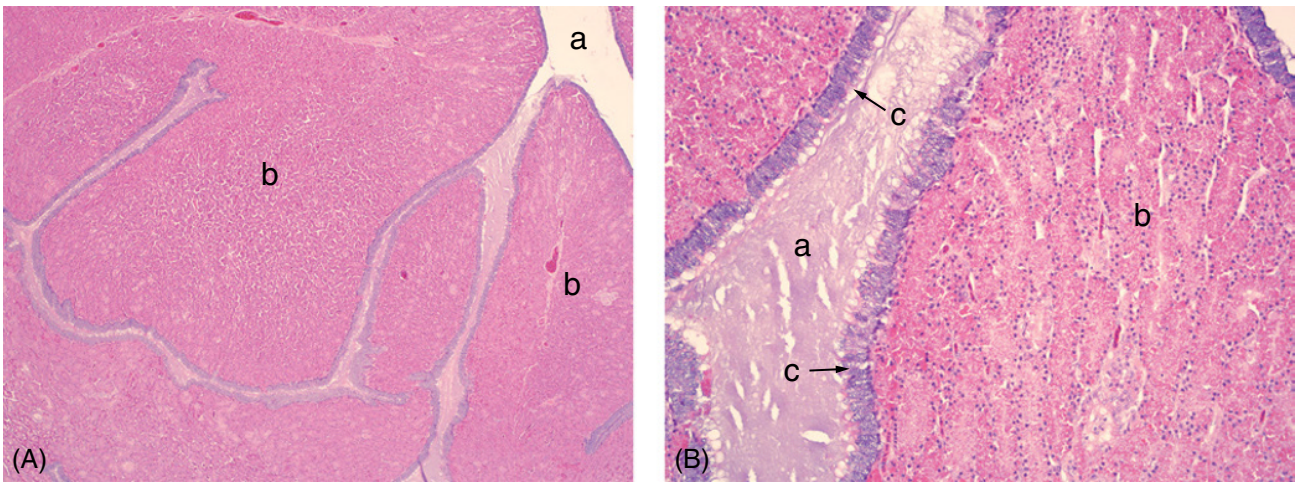

Figure 7.16 Histological section of a hen's magnum wall (A and B). H&E stain. The lumen (a) is narrow and full of proteinaceous secretion, (b) folds of mucosa well developed in height and thickness due to the marked proliferation of the tubular glands, and (c) the mucosa in lined by ciliated simple columnar epithelium with goblet cells.

phases. The main secretion of these glands is ovomucin (ovoalbumen) which is the main component of the egg white.

It is described that chicken produces mainly dense albumen but, while the egg passes through the different segments of the oviduct, water is added transforming it into thin albumen. The proportion of albumen types in a given egg is distributed as follows: thick albumen 57%, central thin albumen 17%, external thin albumen 23%, and chalaza 3% (Poultryhub Australia 2023). However, these percentages may vary considerably depending on the hen's genetics and age, as well as the egg's age and storage conditions.

7.2.2.3 Isthmus

The isthmus is a short segment of the oviduct (about 8–10 cm) with a smaller diameter than the magnum. In fact, the boundary between the magnum and isthmus is clearly distinguished by a narrow-constricted band (1- to 3-mm wide) of tissue called zona translucent, which contains no tubular glands (glandless zone). However, toward the distal part of the isthmus, there is a clear extensive glandular zone. The markedly high mucosal spiral folds from the magnum become reduced in height and thickness toward the end of the magnum and the beginning of the isthmus. Then, the height slowly increases toward the caudal portion while the secondary folds increase in number (Gilbert 1979, pp. 321–324). The surface epithelium is composed of a tall simple columnar epithelium with ciliated and goblet cells. The glandular elements of the caudal isthmus are distinctive and more abundant than in any other segment of the oviduct. These glands are not subjected to phases as in the magnum and secrete different substances that compose different part of the egg such as albumen (up to 20% of the total), water, and the fiber-core proteins that constitute the shell membranes (inner and outer) (see Chapter 15 The Egg Anatomy). The shell membranes separate after the egg is laid and cooled, creating an air chamber or cell at the blunt end of the egg. As the egg ages, the interior contents lose water, and the air cell increases in size being a good indicator of the freshness of the egg and the storage conditions. Despite early controversy, it appears that the first deposits of calcium (but not calcium carbonate) occur in the isthmus (Gilbert 1979, p. 324; King 1993, p. 378, in Baumel et al. 1993). The muscular layer of the wall is more developed and thicker than in the magnum, especially the circular layer.

The developing egg takes approximately 75 minutes to pass through the isthmus to acquire all its new layers.

7.2.2.4 Uterus (Shell Gland, Eggshell Gland)

There is no distinct anatomical boundary between the isthmus and the uterus. The uterus or shell gland is relatively short (8–12 cm in length) and transitions from a tubular portion (red region, pars cranialis uteri) to a pouch-like section (pars major uteri) with a higher diameter. It is in this last pouch-like segment where the forming egg remains the longest in the oviduct (18–20 hours). When the egg reaches the uterus, it is constituted by a central yolk surrounded by a thick portion of the albumen encased in a double layer of soft-shell membranes. The two main events occurring in this segment to this forming egg are the process of plumping, where water is added to the albumen and become thin as well as thick albumen, and the formation of the hard shell (testa) with the deposit of pigment (depending on hen breed) which produce colored shells (see Chapter 15, The Egg Anatomy), hence shell gland.

The hard shell (testa) formation starts in the last part of the isthmus with the deposition of small clusters of calcium carbonate crystals onto the newly formed outer shell membrane. These form the nucleus or core (mammillary core) for the subsequent calcium carbonate deposition in the uterus. The number of initial cores is genetically controlled and determined the final thickness of the shell. The inorganic portion of the testa is formed in two layers: the inner mammillary layer, a sponge like layer composed of soft calcite crystals ($CaCO_3$), and the outer palisade layer, formed by columns of hard calcite crystals. The strength of the shell depends on the thickness of these layers. For more detail, refer to Chapter 15, The Egg Anatomy.

The last region of the isthmus and the uterus are very similar histomorphologically in respect to the epithelium and the glands (Figure 7.17). However, the abundant glands present in this segment have a different function since they secrete calcium carbonate. The mucosa of the last segment of the uterus (recessus uteri) is different than the remaining uterus and shares characteristics of the vagina. The most caudal part of the uterus has a well-developed muscular circular layer that extends onto the circular folds of the mucosa to help with egg expulsion (Figure 7.17). This final portion of the uterus is demarcated by a well-developed muscular utero-vaginal sphincter, a thickening of the circular muscle layer. While the egg is waiting in the uterus during the last 1.5 hours before oviposition, a new thin organic layer, the cuticle, is deposited by the epithelial cells lining the uterus (Nys et al. 1999).

7.2.2.5 Vagina

The vagina is an S-shaped, short (8- to 12-cm long), narrow and muscular tube, which extends from the utero-vaginal sphincter to the cloaca with whom it communicates through a narrow opening (ostium cloacale oviductus sinister). It has a thick wall because of the presence of a thick muscular layer, especially the inner circular layer. The muscle layer is thicker in this segment of the oviduct than in any other regions. The mucosa is composed of primary

Figure 7.17 Histological cross section of a hen's uterus (shell gland). H&E stain. (a) Uterus lumen, (b) mucosa arranged in abundant leaf-shaped folds, well developed circular (c) and longitudinal (d) muscle layers.

and secondary folds covered by simple ciliated and non-ciliated columnar epithelium without glands (Figure 7.18).

The cranial (proximal) portion of the vagina, close to the utero-vaginal sphincter area, contains the spermatic tubules (tubuli spermatici or fossulae spermaticae) or sperm nests. These are tubular spaces in the mucosa that act as sperm storage sites that keep the sperm viable for several weeks after mating. In this way, hens can release fertilized eggs weeks after a single insemination or the presence of the male (Bakst et al. 2010). These structures were formerly called glands but the secretory activity, if any, in the different species of birds is unclear and may not be true glands. Hence, the word "tubule" seems more adequate (Bakst 1987; King 1993, p. 379, in Baumel et al. 1993).

7.2.3 Cloaca

The formed egg can be held in the cloaca for a short period of time prior to being laid. It is believed that the egg usually enters this last segment of the reproductive tract acute pole first, but it usually rotates here, during the transition from urodeum to proctodeum, to be laid blunt pole first given the appropriate time.

7.2.4 The Formation of a Chicken's Egg

The time taken to complete the creation of an egg from ovulation (release of the chicken follicle or yolk) until the egg passes through the vent varies with individuals (age, breed, environmental conditions) but ranges between 23 and 26 hours (Table 7.1). Eggs laying in hens is not necessarily continuous. Groups of 4–6 oocytes develop in a

Table 7.1 Functions of different segments of the hen's oviduct with respect to the formation of the egg.

Segment	Time in transit	Structure formed
Ovary	9–10 days	Yolk (mature follicle)
Infundibulum	15–30 min	Starts the albumen and chalaza
Magnum	2–3 h	Albumen (50%) mainly thick, dense; chalazae
Isthmus	75 min	Albumen (20%); shell membranes
Uterus	18–20 h	Hard eggshell (testa); albumen (30%) mainly thin (plumpling); cuticle
Vagina	15 min–2 h	Cuticle
Cloaca		

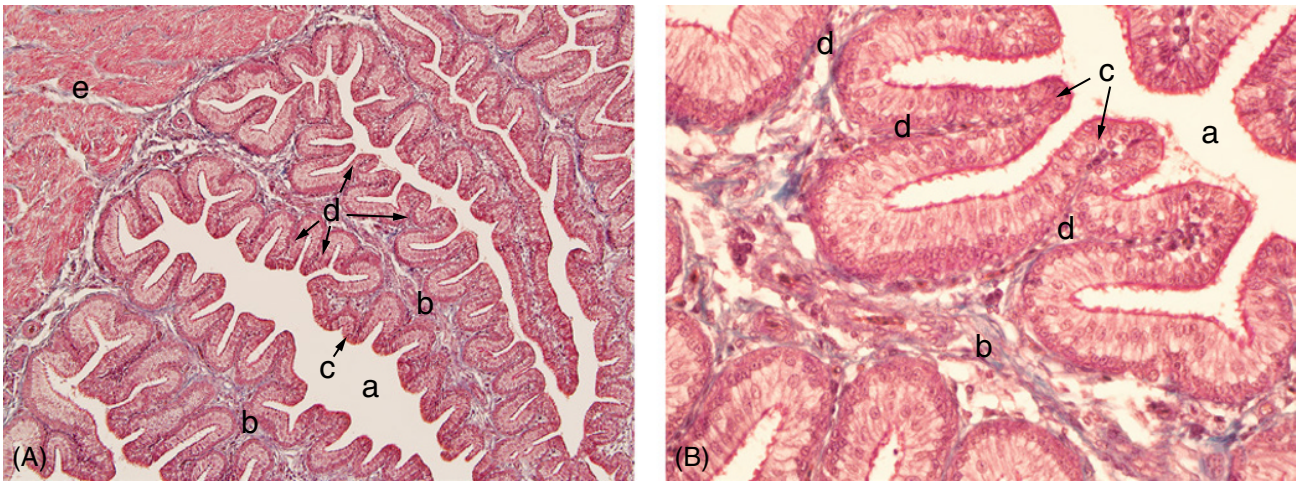

Figure 7.18 Histological cross section of a hen's vagina (A and B). Trichrome stain. (a) Lumen, (b) long and slender mucosal folds with numerous secondary folds (d), (e) thick circular (inner) muscular layer.

hierarchical manner so eggs can be laid daily for four to six days, producing a clutch of eggs. The regular process to form a chicken egg can be split into four steps: creation of a mature yolk, production of the egg white or albumen, formation of the shell membranes, and formation of the testa or hard shell. For a detailed explanation of the chicken egg formation, consult Burley and Vadehra (1989).

7.2.4.1 Yolk

The formation and development of the yolk takes place in the ovary. A 2- to 3-mm primary follicle grows to 4- to 5-cm mature follicle (containing the yellow yolk) in about 10 days initiated by the follicle-stimulating hormone secreted by the anterior lobe of the pituitary gland. All the compounds in the yolk are synthetized in the liver and transported by the blood stream to the target follicle to form the yolk. The yolk is contained in a very thin, transparent membrane called the vitelline membrane and the follicle acts as the container and the source of blood. The presence of a mature yolk in a follicle causes hormones from the ovary to stimulate the release of luteinizing hormone (LH) by the pituitary gland. A mature follicle has an elongated area or band without blood vessels on the surface opposite to its attachment to the ovary. This area is called the stigma (Figure 7.13). It is where the follicle normally splits to release the yolk into the oviduct after a surge of LH. The yolk with the oocyte usually escapes the fibrous capsule (thecal layer) of the follicle at the stigma, but on occasions the follicle ruptures in a different part of the highly vascular theca and will contaminate the yolk with blood spots. The ovulation (release of the yolk and oocyte) triggers the subsequent steps in the formation and laying of the egg.

7.2.4.2 Synthesis of Albumen

Once the yolk is released, it enters the first segment of the oviduct, the infundibulum, where fertilization occurs if there has been sperm deposition. In this segment, the yolk is surrounded by a thin dense mucin-containing secretion in the form of a delicate glycoprotein fiber network and the initial chalaza fibers are seen (Nickel et al. 1977, p. 82). Half of the albumen mass (mainly dense) is acquired in the magnum for 3 hours and the remaining is provided by the transit through the isthmus and uterus (75 minutes and 18–20 hours, respectively). As mentioned in Section 7.2.2.4. Uterus (Shell gland), the addition of water to the secreted albumen (plumping) completes the mixture of the egg white with different types of albumen (thick and thin) as well as the arrangement (outer thin, middle dense, and inner thin). The differentiation of the chalazae, a dense albumen derivative, is also evident after the magnum passage and ends in the isthmus and uterus.

7.2.4.3 Production of Shell Membranes

The formation of the shell membranes starts at the most caudal part of the magnum but is completed in the isthmus which is the main creator of these layers (Burley and Vadehra 1989, p. 20). Both membranes are deposited on top of the albumen during the passage through the isthmus for about 60–75 minutes.

7.2.4.4 Formation of the Testa or Hard Shell

The eggshell or testa formation is the process by which an egg develops a hard, calcified, protective outer layer or shell. As mentioned in Chapter 15 The Egg Anatomy, the shell is composed primarily of calcium carbonate and a small amount of organic material. The testa starts in the isthmus with the mammillary cores, which are the centers of mineralization for the subsequent calcium carbonate deposition in the uterus in a series of thin layers. The growth of the testa is slow during the first 2–3 hours, but then the mineralization proceeds at a rate of 300 mg of Ca per hour (Burley and Vadehra 1989, p. 21). The calcium for the eggshell comes from the diet and the skeleton via blood stream. A hen uses approximately 2.5 g of calcium in the formation of one normal egg. If not enough calcium is acquired by the diet each day (less than 2.0 g per day), it is essential to mobilize more skeletal calcium to make up the deficit.

The calcium carbonate is arranged in a lattice-like structure that gives the shell its strength and durability. At this stage, the shell pigments are deposited in the surface region of the shell. As a curiosity, chickens with red earlobes tend to lay brown eggs and those with white earlobes tend to lay white eggs (Morishita et al. 2021). The testa is formed in about 20 hours, and during this time the egg rotates inside the uterus to ensure that the shell is deposited evenly around the egg. During the last 1–2 hours in the uterus and after, the testa is fully formed the cuticle is deposited by the epithelial cells lining the mucosa (Nys et al. 1999).

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8

Endocrine System

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8.1 Introduction

8.1.1 Chicken Endocrine System: A Key Regulator of Physiological Processes

The endocrine system plays a crucial role in regulating various physiological processes in organisms. It consists of a complex network of glands that produce and release hormones, as well as scattered endocrine cells within the tubular organs which act as chemical messengers to communicate and coordinate activities throughout the body. This chapter aims to provide an overview of the avian endocrine system, its major components, its anatomy, histology, and significance in maintaining homeostasis and ensuring the proper functioning of numerous organs.

The endocrine system comprises various glands distributed throughout the body, including the hypothalamus, pituitary, thyroid, and adrenal glands, in addition to the pancreas, reproductive organs, and scattered endocrine cells within several tubular systems among others.

External stimuli, including social factors, temperature, food availability, and sunlight cause the endocrine system to release hormones (Hiller-Sturmhöfel and Bartke 1998). The hormones secreted into the bloodstream need to travel to target tissues and organs where they exert their effects (DuBray La Perle and Jordan 2012). The endocrine system works slower than the nervous system. Hormonal signaling and communication hormones, chemical messengers of the system, are classified into different categories, including peptide, steroid, and amino acid-derived hormones. These hormones bind to specific receptors on target cells to initiate a cascade of intracellular events that regulate cellular function and gene expression. Examples of key hormones are insulin, cortisol, thyroid, growth hormone, testosterone, and estrogen. The endocrine system plays a crucial role in the regulation of metabolism. Thyroid hormones influence the metabolic rate and energy expenditure. The interplay of various hormones, such as insulin, glucagon, and cortisol, ensures the proper utilization and storage of nutrients thus maintaining energy balance (Fliers 2006). The endocrine system is closely intertwined with reproductive functions. The hypothalamic-pituitary-gonadal axis, involving the hypothalamus, pituitary gland, and gonads (testes and ovaries) regulates the production of sex hormones (testosterone and estrogen). These hormones govern sexual development, fertility, and reproductive processes (Schneider 2004). Growth hormone (GH) secreted by the pituitary gland plays a central role in growth and development. GH promotes cellular growth, protein synthesis, bone development, and muscle growth. The endocrine system coordinates the body's response to stress and facilitates adaptation. The hypothalamic-pituitary-adrenal (HPA) axis is activated during stress, resulting in the release of cortisol from the adrenal glands. Cortisol helps mobilize energy reserves, suppresses immune function, and enhances the body's response to stressors. Clinical implications and pathological disruptions or dysfunctions within the endocrine system can give rise to a range of disorders. Hormonal imbalances can impact reproductive health, and growth disorders, and contribute to the development of certain syndromes and cancers (Smith et al. 2019).

Birds possess analogous counterparts to most hormones and glands described in mammals, which often fulfill similar roles (Scanes and Sturkie 2015). Avian hormones exhibit distinctive functions specific to avian traits such as egg-laying production, seasonal adaptation, and crop milk production that allows certain bird species to provide parental care and nourishment to their young. The salt gland is a remarkable adaptation that enables marine birds to survive and thrive in saline environments by efficiently regulating their water and

Anatomy and Histology of the Domestic Chicken, First Edition. Edited by Wael Khamas and Josep Rutllant.

electrolyte balance, migration, and vocalization (Scanes and Sturkie 2015). Moreover, there are notable variations in metabolic processes between birds and mammals. While not all protein/peptide hormones and neuropeptides identified in mammals are present in birds, the avian system expresses a few unique protein/peptide hormones, like the prolactin-like protein (PLP), absent in mammals. Birds exhibit various reproductive and physiological adaptations that involve the expression of PLP. It is believed to play a role in the development and maintenance of brood patches, which are specialized featherless areas on the abdomen used for incubating eggs, as well as the initiation and regulation of incubation behaviors in both males and females (Buntin 1986).

Certain external stimuli could influence the endocrine system through simultaneous effects on multiple glands. Recent studies have shown that implementing specific lighting schedules for broiler chickens can have beneficial effects on their overall well-being and health outcomes (Wu et al. 2022). De Oliveira and Lara (2016) demonstrated that manipulating the light regimen can stimulate feed intake in broilers, potentially leading to improved growth. Additionally, Hajrasouliha and Kaplan (2012) found that appropriate lighting conditions can modulate the systemic immune response in broiler chickens, potentially enhancing their immune function and disease resistance. Moreover, Parvin et al. (2014) discovered that optimized lighting regimens can help reduce physiological aggressive behaviors in broilers, promoting a calmer and less stressful rearing environment. However, it is important to note that not all physiological processes in birds are responsive to external stimuli. One specific example is the regulation of circadian rhythms, which seem to maintain their consistency even when researchers manipulate the light levels and timing. Despite alterations in the light-dark cycles and exposure to constant light or constant darkness, the underlying circadian rhythms remained relatively stable (Foley et al. 2011).

8.2 Physiological Balance

The endocrine glands in birds exhibit intricate interactions and integration to maintain overall physiological balance. Feedback mechanisms, neural connections, and hormonal crosstalk contribute to the coordination of hormone production and regulation in response to internal and external stimuli (Wingfield et al. 1998). Numerous hormones in birds play essential roles within feedback loops, which have evolved to maintain homeostasis and ensure the balance of chemical processes. The intricate feedback mechanisms involving hormones are crucial for maintaining physiological equilibrium in birds. These mechanisms have evolved over time to enable birds to adapt to their environment and cope with various challenges.

8.2.1 Homeostasis

The endocrine system is an intricate and indispensable regulatory system in maintaining homeostasis and orchestrating a wide array of physiological processes. Through the secretion of hormones, it influences metabolism, growth, reproduction, stress responses, and more. This chapter provides an overview of the endocrine glands in birds, with specific attention to chickens. It covers the major endocrine glands. It explores their anatomical and histological characteristics, hormone production, and functions in avian species, highlighting their significance for growth, development, reproduction, and overall health. Understanding the endocrine system in birds, especially chickens, is essential for optimizing poultry production, management practices, health, and welfare. Influencing hormone levels, such as thyroid hormones or sex hormones, can impact growth, reproduction, and overall performance in poultry (Etches 1996). Understanding the complexities of the endocrine system is also essential for advancing medical treatments and interventions for endocrine-related disorders.

8.3 Major Endocrine Glands

The major endocrine glands whose anatomy, histology, and physiology will be described in detail are:

1) **Hypothalamus**: The hypothalamus plays a key role in regulating hormone secretion through its control over the pituitary gland. It releases various neurohormones that influence the secretion of hormones involved in growth, reproduction, and stress response.
2) **Pituitary gland (hypophysis)**: The pituitary gland is often referred to as the "master gland". It is located at the base of the brain and divided into two lobes (anterior and posterior). It produces and releases a multitude of hormones that regulate growth, metabolism, reproduction, and osmoregulation in birds (Fusani 2008).
3) **Thyroid glands**: The thyroid glands, located in the neck region in mammals and at the entrance of thoracic-pleural cavity in birds, synthesize thyroid hormones that are crucial for regulating metabolism, growth, and development. These hormones influence thermoregulation, energy balance, and overall physiological homeostasis in birds (Bohler et al. 2021).
4) **Adrenal glands**: The adrenal glands, situated near the kidneys, produce glucocorticoids and mineralocorticoids that are involved in stress response, immune function, electrolyte balance, and energy metabolism in birds (Puvadolpirod and Thaxton 2000).
5) **Pancreas**: The pancreas serves both exocrine and endocrine functions in birds. It produces digestive enzymes

and hormones, including insulin and glucagon, which regulate glucose metabolism and energy utilization (Ku et al. 2000). These hormones play critical roles in maintaining blood glucose levels and energy homeostasis in birds (Dunning et al. 2005).

6) **Gonads**: The gonads (testes and ovaries) are responsible for reproductive functions. In addition to their exocrine function, liberating the gametes, they produce sex hormones (androgens and estrogens), which govern sexual development, behavior, and reproductive cycles.
7) **Pineal gland**: The pineal gland is located above the brain, thus epiphysis cerebri is another name for it. It synthesizes and releases melatonin hormone. It plays a crucial role in regulating circadian rhythms, seasonal adaptations, and photoperiodic responses in birds (Loredana and Solcan 2023).
8) **Ultimobranchial body and carotid body.**
9) **Scattered endocrine cells**: Various tissues in the body, once considered solely as targets for hormones, are also capable of producing biologically active proteins and peptides. Examples include adipose tissue (adiponectin and leptin), heart cells (natriuretic peptides), liver (insulin-like growth factor-1), kidneys (renin which results in the production of angiotensin-I and angiotensin-II, as well as 1,25-dihydroxy vitamin D3 and erythropoietin), and endocrine cells in the gastrointestinal tract (Scanes and Sturkie 2015).

8.3.1 Hypothalamus

The hypothalamus in chickens is a small, but fundamental structure, key regulatory center in the brain that plays a crucial role in regulating various physiological processes. As a part of the hypothalamus-pituitary axis (HPA), the hypothalamus produces and releases various neuropeptides that influence the secretion of pituitary hormones. This interaction between the hypothalamus and pituitary gland regulates numerous important physiological processes. It serves as a crucial interface between the central nervous system and the endocrine system, integrating environmental cues and internal signals to maintain optimal physiological balance. The hypothalamus communicates with the pituitary gland through a specialized portal system (hypophyseal portal circulation). This system allows hypothalamic hormones to be directly transported to the anterior pituitary thus regulating the release of pituitary hormones (Scanes and Sturkie 2015).

8.3.1.1 Anatomy

In chickens, the hypothalamus is in the ventral part of the diencephalon and is divided into several distinct nuclei. These nuclei include the preoptic, anterior hypothalamic, mediobasal hypothalamus, and posterior hypothalamus. Key nuclei within the avian hypothalamus include the preoptic area (POA), paraventricular nucleus (PVN), and arcuate nucleus (ARC). Each region of the hypothalamus contributes to different aspects of avian physiology and behavior.

Few prominent nuclei in the chicken hypothalamus include the following:

1) **Preoptic nucleus:** It is located at the anterior part of the hypothalamus. This nucleus is involved in thermoregulation and reproductive behaviors in chickens (Bahhazart et al. 1996).
2) **PVN:** This nucleus is responsible for the synthesis and release of neurohormones, including oxytocin and arginine vasotocin, which play important roles in regulating social behaviors and reproductive processes (Balthazart and Ball 2007).
3) **Suprachiasmatic nucleus (SCN):** It is found in the anterior part of the hypothalamus to serve as the master circadian clock, controlling daily rhythms and regulating various physiological processes based on light cues (Yoshimura et al. 2001).
4) **Arcuate nucleus:** This nucleus is involved in regulating appetite and feeding behaviors. It contains neurons that produce key appetite-regulating hormones, such as neuropeptide Y and pro-opiomelanocortin.

8.3.1.2 Histology

The following are some key cell types commonly found in the hypothalamus of birds, including chickens (Saper 2002):

1) **Neurons:** Neurons are the primary cell type in the hypothalamus responsible for transmitting and processing signals. They form intricate networks and are involved in regulating various physiological functions.
2) **Glial cells:** Glial cells, including astrocytes and oligodendrocytes, provide support and insulation to neurons. They play important roles in maintaining the structural integrity of the tissue and facilitating neuronal communication.
3) **Secretory cells:** The hypothalamus contains specialized secretory cells that produce and release neuropeptides and hormones. These cells are involved in regulating various processes such as reproduction, feeding behavior, and stress response.
4) **Endothelial cells:** Blood vessels in the hypothalamus are lined with endothelial cells, which help supply nutrients and oxygen to the tissue and facilitate the exchange of molecules between the blood and brain.

8.3.1.3 Function

The hypothalamus is a highly complex structure comprising several nuclei that secrete neuropeptides and regulate the release of various hormones from the anterior

pituitary gland (adenohypophysis), influencing the secretion of hormones such as gonadotropins, prolactin, and corticosteroids. It regulates the release of neurohypophysial hormones, including vasopressin and oxytocin, from the posterior pituitary gland. In birds, oxytocin plays a role in the release of the yolk. The quantity of the releasing factors and oxytocin released is influenced by day length, among other external stimuli (Cassone 2014). The longer the day is, up to 18 hours, the greater the amount of releasing factors released and the greater the effect on the target gland or function. Other hypothalamic factors, such as thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone, play roles in regulating thyroid and adrenal functions, respectively (Leung et al. 1984).

The hypothalamus in chickens is responsible for controlling temperature regulation, feeding behavior, water balance, and reproduction. It plays a vital role in maintaining homeostasis. It regulates body temperature through thermoregulatory mechanisms, ensuring that birds maintain a stable internal temperature. It is involved in the control of various behaviors, including feeding, drinking, aggression, sexual behavior, and parental care. Different hypothalamic nuclei, such as the lateral and the ventromedial hypothalamic nuclei, are associated with specific behavioral patterns (Balthazart and Ball 2007). It controls feeding behavior in chickens by regulating hunger and satiety. The arcuate nucleus in the hypothalamus produces neuropeptides that stimulate or suppress appetite, depending on the nutritional status of the chicken (Sohn 2015). Environmental cues, such as light, temperature, and social interactions, can influence hypothalamic function in chickens. Photoperiod, for instance, affects reproductive activities and seasonal adaptations in response to changing day lengths (Bentley 2009). The hypothalamus regulates water balance in chickens by controlling thirst and fluid intake. The supraoptic nucleus produces vasopressin which regulates water reabsorption in the kidneys.

The hypothalamus plays a key role in regulating reproductive functions in chickens. It controls the secretion of gonadotropin-releasing hormone from the POA and the mediobasal hypothalamus, which governs the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland (Bédécarrats et al. 2006). The avian hypothalamus-pituitary axis is tightly regulated by feedback mechanisms to maintain hormonal balance. Negative feedback loops involve peripheral glands or target organs, such as gonads or thyroid gland, which influence the hypothalamus and pituitary gland to modulate hormone secretion (Wingfield et al. 1998).

8.3.2 Pituitary Gland (Hypophysis Cerebri)

In chickens, the hypophysis (pituitary gland) is a small, oval-shaped gland located at the base of the brain, well protected by the surrounding skull bones. It plays a critical role in regulating many important physiological processes, including growth, metabolism, reproduction, and stress response. The pituitary gland orchestrates the initiation or cessation of hormone secretion from other glands within the body. In birds, the pituitary gland is particularly involved in controlling reproductive processes, such as yolk production and egg deposition, as well as growth. Moreover, it stimulates the thyroid gland, pineal gland, and islets of Langerhans. Among the hormones secreted by the anterior pituitary gland in chickens are FSH, LH, GH, adrenocorticotropic hormone (ACTH), and prolactin (PRL) (Kaneda et al. 2018).

8.3.2.1 Anatomy

The pituitary gland is connected to the hypothalamus by the infundibulum. It is divided into two main parts: the anterior pituitary (adenohypophysis) and the posterior pituitary (neurohypophysis). The anterior pituitary is stimulated by special releasing hormones from the hypothalamus of the brain to produce and release GH, prolactin, thyroid-stimulating hormone (TSH), adrenocorticotrophic, FSH, and LH, which are involved in the regulation of growth, development, and reproduction.

The posterior pituitary stores and releases two hormones produced by the hypothalamus: oxytocin and vasopressin (antidiuretic hormone, ADH). Oxytocin plays a role in the release of the yolk into the oviduct and the actual laying of the egg or oviposition. ADH acts on the kidney collecting ducts and positively affects the reabsorption of water. These hormones are involved in regulating water balance, blood pressure, and social behavior in chickens.

8.3.2.2 Histology

The adenohypophysis can be divided into three main regions: pars distalis (main secretory portion), pars intermedia, and pars tuberalis (adjacent to the infundibulum). Pars distalis, the largest and most prominent region of the chicken hypophysis, consists of various cell types, including acidophils, basophils, and chromophobes (Figure 8.1A and B). Acidophils secrete GH and prolactin (PRL), while basophils secrete ACTH, FSH, and LH (Puebla-Osorio et al. 2002). Chromophobes are considered inactive or non-secretory cells. Pars intermedia, located between pars distalis and pars nervosa, contains melanotrophs that produce melanocyte-stimulating hormone (MSH) (Saneyasu et al. 2011). Melanotrophs are responsible for regulating pigmentation and other physiological processes related to

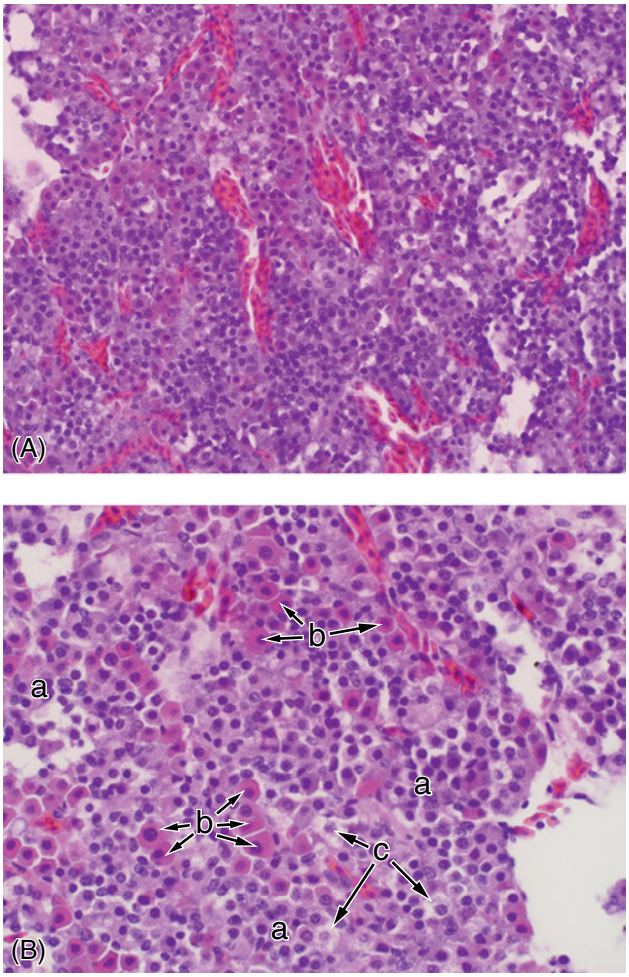

Figure 8.1 (A, B) Histological section of the chicken adenohypophysis showing the highly vascular region and several cell types, (a) basophils, (b) eosinophils, and (c) chromophobes. H&E stain.

coloration in chickens. Pars tuberalis, situated around the infundibulum, consists of specialized cells associated with neuroendocrine functions. It contains folliculo-stellate cells that support the surrounding endocrine cells and may have a role in hormone release regulation (Inoue et al. 1999).

8.3.2.3 Adenohypophysis Cells

1) **Acidophils:** These cells stain with acidic dyes and are responsible for the production of GH and PRL hormones.
2) **Basophils:** These cells stain with basic dyes and produce TSH, ACTH, FSH, and LH.
3) **Chromophobes:** These cells do not stain readily with either acidic or basic dyes and their function is not fully understood. They are either inactive cells (degranulated) or transitional cells.
4) **Colloid-filled follicles:** The pars intermedia region of the adenohypophysis may contain colloid-filled follicles, which are specialized structures involved in the production and release of MSH.
5) **Blood vessels:** The adenohypophysis is highly vascularized, with numerous special blood vessels supplying the gland to facilitate hormone secretion and distribution.

8.3.2.4 Neurohypophysis

In chickens, neurohypophysis primarily consists of nerve fibers with specialized glial cells known as pituicytes. Pituicytes are the predominant cell type found in this region and provide structural support to the gland. Nerve fibers originate from the hypothalamus and extend into the neurohypophysis through their axon terminals. Axon terminals contain secretory vesicles (Hering bodies), which store hormones produced by the hypothalamus, such as oxytocin and vasopressin (ADH). Pituicytes have elongated cell bodies and processes that surround the nerve fibers, creating a network within the gland. As glial cells, they interact closely with the nerve fibers and are involved in various functions, including the regulation of fluid balance and the release of hormones (Hatton 1988).

8.3.2.5 Function

The pituitary gland is responsible for the synthesis and secretion of various hormones that control essential physiological processes in chickens. These hormones include GH, FSH, LH, PRL, and ACTH, among others. These hormones act on target tissues to regulate growth, development, metabolism, and reproductive functions.

Secreted GH stimulates protein synthesis, cell proliferation, and skeletal growth, ultimately influencing body size and weight. The pituitary gland plays a central role in regulating the reproductive functions of chickens. FSH and LH, produced by the anterior pituitary, are essential for follicular development, ovulation, and the production of sex hormones such as estrogen and progesterone. PRL, secreted by the anterior pituitary, is involved in metabolic regulation in chickens. It promotes broodiness, which is characterized by changes in behavior and physiology associated with incubation and maternal care (Mohamed et al. 2016). PRL also influences water balance, osmoregulation, and calcium metabolism in birds. Environmental factors such as photoperiod, temperature, and social cues can influence pituitary function in chickens. Photoperiod plays a critical role in regulating seasonal changes in reproductive hormones and behavior. Temperature variations can also affect pituitary hormone secretion and reproductive performance in chickens (Lara and Rostagno 2013).

Disorders of the pituitary gland in chickens can lead to reproductive abnormalities, growth disturbances, and

metabolic imbalances. Pituitary tumors, for example, can cause excessive hormone production or disrupt hormone secretion, resulting in reproductive dysfunction or growth abnormalities (Melmed 2003).

8.3.3 Adrenal Gland

In chickens, the adrenal medulla has been shown to be involved in the regulation of various physiological processes, including body temperature, feed intake, and energy metabolism (Liu et al. 2018). Additionally, the expression of genes related to catecholamine biosynthesis and signaling has been shown to be affected by various stressors, such as heat stress and immune challenge (Liu et al. 2019).

8.3.3.1 Anatomy

Chickens, like most birds, have two adrenal glands approximately 9-mm long located on each side of the median plane just anterior to the bifurcation of the caudal vena cava, close to the gonads and the anterior division of the kidneys (Wells and Wight 1971). The adrenal glands are small, pyramidal-shaped and produce hormones that principally help the chicken's body response to stress and regulate its metabolism. These glands often exhibit bilateral asymmetry in terms of size and shape, and in some species, such as whitehead sea-eagle and crane, they may even be fused together in the midline (Zhang 1988). In the chicken, there may be small variations in weight, length, width, and thickness of the right and left adrenal glands (Humayun et al. 2012).

8.3.3.2 Histology

The development of the adrenal glands in birds involves two different germ layers: the steroidogenic "inter-renal" tissue, equivalent to the cortex in mammals, arises from the mesoderm, while the catecholaminergic chromaffin tissue, equivalent to the medulla in mammals, originates from neural crest cells. In chickens, there is no clear distinction between the cortex and medulla like in mammals (Kober et al. 2010). The adrenal gland has therefore two cell groups, cortical and medullary cells. These two components extensively intertwine, with the chromaffin tissue (medullary cells) usually occurring as islets amidst cords of steroidogenic cells (cortical cells) (Figure 8.2A–B). However, in certain bird species, the chromaffin tissue tends to concentrate more toward the outer region of the glands (Hartman et al. 1947). Groups of cortical and medullary cells are distributed not uniformly inside the gland and the proportion of cortical to medullary tissues differs in different birds (Tang et al. 2009). In adult chickens, the adrenal cortico-medullary ratio is approximately 1.6:1

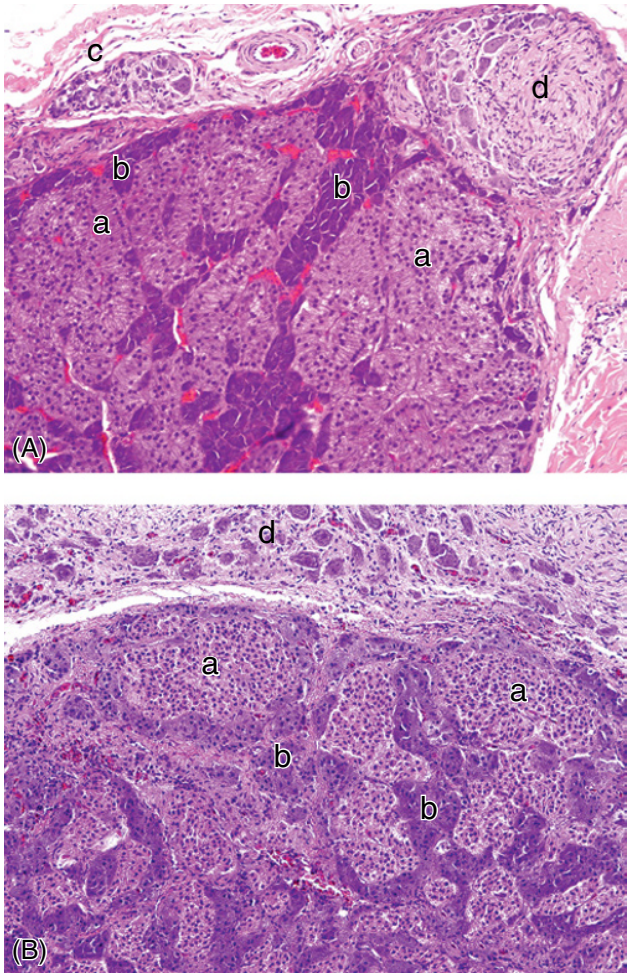

Figure 8.2 (A, B) Histological section of chicken adrenal cortical and medullary tissues, which are intermingled. (a) Eosinophilic cortical, (b) basophilic medullary tissues arranged in islets, (c) connective tissue capsule, and (d) autonomic ganglion. Cortical cells are columnar with a small, round to slightly oval, eccentric nucleus. Medullary cells are polygonal with large, spherical, centrally located nuclei. H&E stain.

(Humayun et al. 2012). The functional significance of higher cortical tissues may be the need for increased production of adrenal cortical hormones (glucocorticoids and mineralocorticoids) in the chicken that, for instance, lives in areas where water conservation is important (Humayun et al. 2012; Moawad and Randa 2017).

In ducks (*Anas*), the cortex of the adrenal gland exhibits cytological differences, suggesting the presence of an outer zone produces aldosterone and an inner zone produces corticosterone, like mammals (Holmes and Cronshaw 1984). The chromaffin cells in the adrenal glands can be histochemically classified into norepinephrine- and epinephrine-producing cells, akin to mammals. The percentage of these cell types can vary significantly among species. For example, the cormorant

(*Phalacrocorax niger*) has 100% norepinephrine cells, while certain passerine birds have 95% epinephrine cells (Ghosh 1980).

8.3.3.3 Function

In chickens, as in most animals, the adrenal gland plays an important role in maintaining homeostasis, or the balance of the body's internal environment. For example, during times of stress, the adrenal gland produces glucocorticoids that help the chicken cope with the stressor by increasing heart rate and respiration and tab into energy stores to provide a burst of energy.

The cortical cells of the adrenal gland produce corticosterone that facilitates carbohydrate and fat metabolism, the breakdown of protein, and plays an important role in a bird's reaction to stress and immune function. Aldosterone, the main mineralocorticoid hormone, increases reabsorption and retention of sodium and 8-hydroxycorticosterone. The expression of genes related to steroid hormone biosynthesis and metabolism is significantly different between laying hens and broiler chickens, indicating that the adrenal cortex plays a role in the regulation of egg-laying performance (Wang et al. 2017).

The medullary cells produce catecholamines, including adrenaline which controls blood pressure, and noradrenaline that regulates fat metabolism. Both hormones are involved in the body's "fight or flight" response to stress, increasing heart rate, blood pressure, and respiratory rate to prepare the bird for action.

8.3.4 Thyroid Gland

8.3.4.1 Anatomy and Histology

The thyroid gland in chickens is located at the entrance of thoracic-pleural cavity of the coelom. It consists of two reddish-purple glands. The size and shape of the thyroid glands in avian can vary depending on the species and developmental stage.

Avian thyroid tissue develops from the endoderm of the pharynx, following a similar pattern observed in other vertebrates (Ramanof 1960). However, unlike mammals, all birds, including chickens, have two distinct reddish-purple glands located on either side of the base of the neck, near the common carotid artery and jugular vein (Astier 1980).

The thyroid glands in birds are externally covered by a thin connective tissue capsule (Figure 8.3A and B). The parenchyma of the thyroid gland consists primarily of follicular cells, as is the case in many bird species. However, in some species, parathyroid tissue and parafollicular (C cells) may also be present within the thyroid gland (Figure 8.4). The histological and physiological characteristics of the follicular epithelium in the avian thyroid gland appear to be similar to those observed in other vertebrates (Astier 1980).

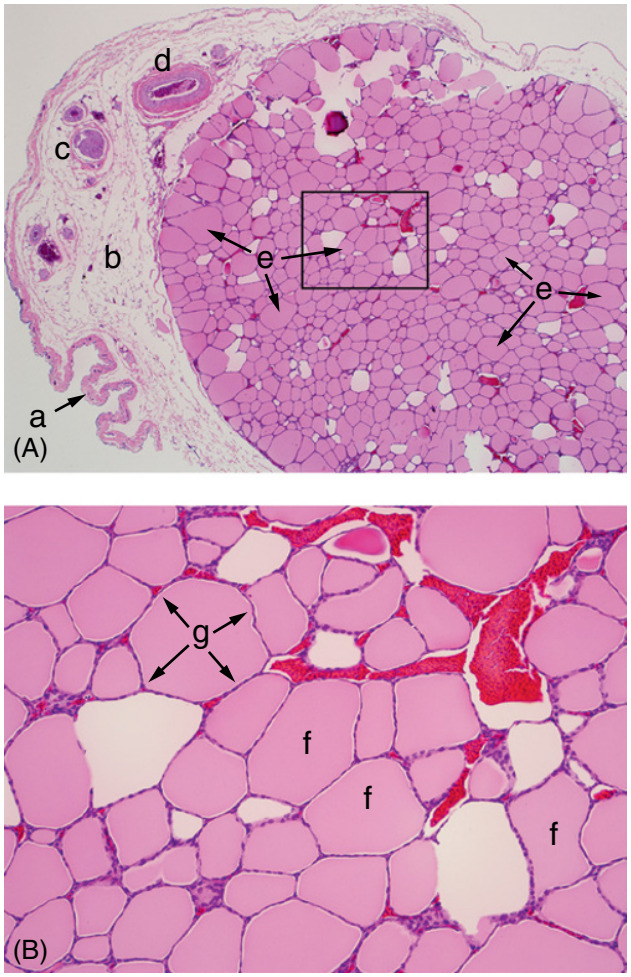

Figure 8.3 Histological section of the thyroid gland (A) and inset (B). (a) Air sac lining epithelium adjacent to (b) connective tissue, (c) nerve fibers, (d) small muscular artery, (e) thyroid follicles, (f) follicular colloid, and (g) thyroid follicular epithelium. H&E stain.

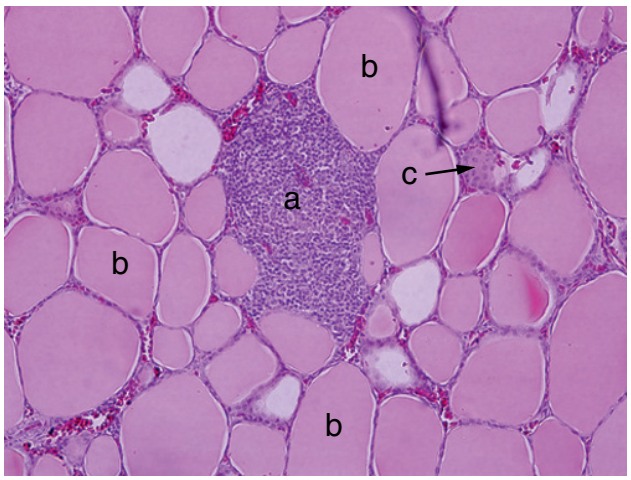

Figure 8.4 Histological section of the thyroid gland of a chicken with part of parathyroid IV (a) embedded inside the thyroid gland, surrounded by thyroid follicles (b) and showing a group of parafollicular (c) cells which are rarely observed in chicken. H&E stain.

Each lobe of the gland consists of numerous follicles composed of follicular cells that synthesize and store thyroid hormones (T3 and T4) (Darras et al. 2006) (Figure 8.3). These follicles are spherical or irregularly shaped structures lined with a layer of follicular cells. A larger follicle size is typically associated with increased thyroid activity. Within the follicles, the cells produce a gel-like substance called colloid. The colloid contains thyroglobulin, a protein precursor to thyroid hormones. Thyroid hormones are synthesized by incorporating iodine into thyroglobulin within the colloid. When stimulated, the follicular cells release stored thyroid hormones into the bloodstream.

8.3.4.2 Function

Thyroid hormones, such as thyroxine (T4), help regulate heat production, carbohydrate metabolism, and promote growth, while triiodothyronine (T3), is essential to promote the growth of skin, and feathers and may be involved in the molting process. Both hormones play a crucial role in regulating many physiological processes in chickens, including growth and development, thermoregulation, energy metabolism, and reproduction (Chowdhury et al. 2021). Thyroxine helps regulate heat production, and carbohydrate metabolism, and promotes high blood sugar level and growth. Thyroid hormones influence various target tissues, including the liver, heart, skeletal muscles, and the central nervous system, through binding to specific nuclear receptors (Darras et al. 2006). The production and secretion of thyroid hormones are controlled by the hypothalamus-pituitary-thyroid axis. The hypothalamus produces TRH, which stimulates the pituitary gland to produce TSH. TSH then stimulates the thyroid gland to produce and secrete thyroid hormones.

In chickens, thyroid hormones also play a vital role in regulating egg production. They are essential for the development and maintenance of skeletal muscles and the cardiovascular system (Karlsson et al. 2015). Additionally, thyroid hormones are involved in regulating body temperature, feather development, and the maturation of reproductive organs in chickens. Thyroid hormone levels can be affected by various factors, including diet, stress, and environmental conditions. Ambient temperature, for instance, affects thyroid hormone metabolism, with higher temperatures often leading to decreased thyroid hormone. Photoperiod plays a crucial role in regulating seasonal changes in thyroid function and reproductive processes in chickens (Dawson et al. 2001). Proper nutrition, including sufficient iodine intake, is essential for normal thyroid hormone synthesis (McNabb 2007).

Diseases affecting the thyroid gland in chickens, such as thyroiditis and hypothyroidism or hyperthyroidism, can have significant impacts on chicken health and productivity. Hypothyroidism is associated with reduced metabolic rate, impaired growth, and reproductive problems (McNabb 2007).

8.3.5 Parathyroid Glands

8.3.5.1 Anatomy and Histology

The parathyroid glands are small, round, yellowish-white glands located at the base of the thyroid glands at the base of the neck. In chickens, there are typically four parathyroid glands, two on each side of the thyroid gland. However, variations in the number and location of these glands have been observed. The cranial and caudal parathyroids originate from the third and fourth pharyngeal pouches, respectively, thus other names like parathyroid III and IV are used. In chickens, the capsules of two parathyroids may be connected to each other, or the tissue itself may be fused within the same capsule (Abdel-Magied and King 1978). In some species, the carotid body as a chemoreceptor is associated with detection of blood gases and can be found near or embedded within the parathyroid glands (Watzka 1933).

The parathyroid parenchyma in birds typically contains two types of chief cells. Chief cells are responsible for synthesizing and secreting parathyroid hormone (PTH), whereas the function of oxyphil cells remains less understood or even absent. The innervation of the parathyroid glands in birds is robust and is primarily associated with blood vessels (Clark et al. 1986).

Accessory parathyroid glands are not consistently found in birds. However, in certain bird species like Gallus (Hodges 1974), Corvus, Strigidae, and Columba (Watzka 1933), accessory parathyroid tissue has been positively identified within the ultimobranchial gland.

8.3.5.2 Function

The primary function of the parathyroid glands is to regulate calcium homeostasis. The secretion of PTH is stimulated by low levels of ionized calcium in the blood. PTH acts on bones, kidneys, and intestines to increase calcium levels. PTH increases the amount of calcium released from bones and absorbed from the intestines. This hormone also stimulates the kidneys to excrete less calcium in the urine. In chickens, the parathyroid glands are also involved in regulating phosphate levels, acid-base balance, and vitamin D metabolism (Jacquillet and Unwin 2019).

Maintaining proper calcium levels is important for the health and survival of chickens, as calcium is a key component in the formation of eggshells. During egg production, hens experience increased calcium demand, which is efficiently managed by the parathyroid glands. When dietary calcium intake is insufficient, the parathyroid glands increase PTH secretion, leading to increased bone resorption and enhanced intestinal calcium absorption (Whitehead

and Fleming 2000). These mechanisms ensure a steady supply of calcium for eggshell formation. If calcium levels are too low, the shells may be thin or malformed.

Environmental factors, such as photoperiod and diet composition, can influence the function of the parathyroid glands in chickens. Research suggests that longer periods of light exposure, characteristic of commercial poultry production, may have a suppressive effect on PTH secretion (Yamamoto 2016). Additionally, dietary imbalances, especially inadequate calcium, or phosphorus levels, can disrupt parathyroid function and lead to skeletal disorders (Whitehead and Fleming 2000). Hypocalcemia, commonly known as "egg-laying fatigue," is a condition characterized by low blood calcium levels, leading to muscle weakness, seizures, and reduced eggshell quality. Hyperparathyroidism can cause excessive bone resorption, leading to weakened bones and increased fracture risk.

8.3.6 Ultimobranchial Bodies

The ultimobranchial bodies (glands) (UB) have an important role in calcium homeostasis and bone development, with implications for poultry management, particularly in optimizing skeletal health and eggshell quality in chickens.

8.3.6.1 Anatomy and Histology

In non-mammalian vertebrates, including birds, C cells that secrete serum calcium-lowering hormone, calcitonin, are not incorporated within the thyroid gland but are in a separate organ, the ultimobranchial gland. The structure, cellular composition, and position of the ultimobranchial gland can vary among different species. In chickens, there is a series of endocrine organs, including the thyroid gland, cranial and caudal parathyroid glands, carotid body, and ultimobranchial gland (a 1–3 mm long gland), situated between the vagus nerve and its branch, the recurrent laryngeal nerve, at the cervicothoracic junction (Figure 8.5A and B). These organs receive arterial blood supply from a single trunk originating from the common carotid artery (Kameda 1990). In contrast to the thyroid gland in mammals, the ultimobranchial gland in chickens is densely innervated by nerve fibers from both the vagus and recurrent laryngeal nerves (Kameda et al. 1988).

The avian ultimobranchial gland develops from two distinct components: (i) endodermal outpocketings of the pharynx and (ii) invasion of calcitonin-producing C cells derived from the neural crest (Le Douarin et al. 1974). The resulting structure can vary among different species (Watzka 1933), and the presence of both components close to adjacent structures makes it challenging to easily define the organ. Therefore, the term "ultimobranchial body" can be misleading. In species like columbids (pigeons and doves), C cells have been found

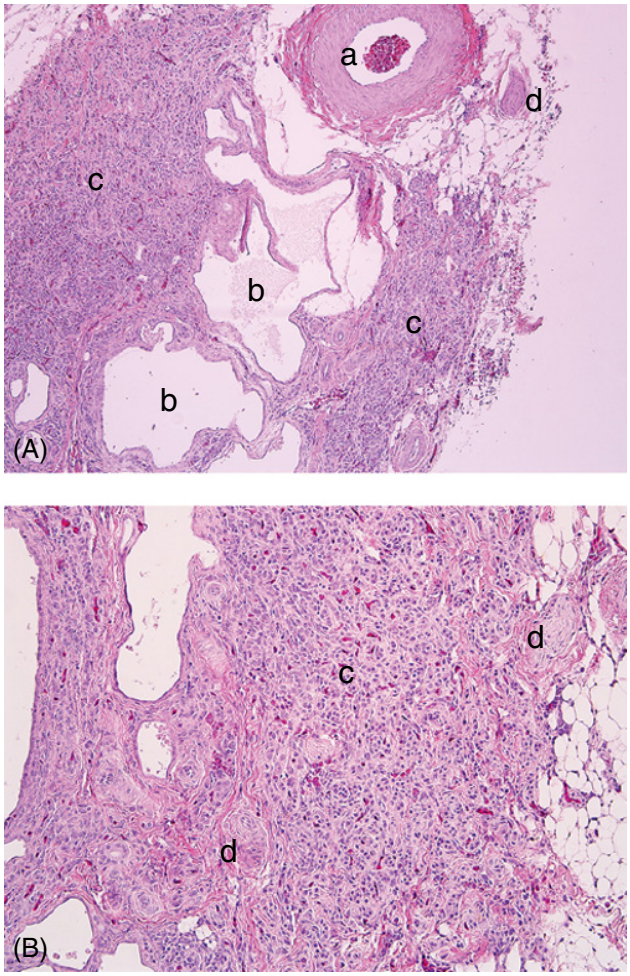

Figure 8.5 (A, B) Chicken ultimobranchial body close to the bifurcation of the brachiocephalic artery. (a) Muscular artery (subclavian), (b) venous plexus, (c) ultimobranchial body, and (d) nerve fibers. H&E stain.

within the thyroid gland, resembling the situation seen in mammals (Stoeckel and Porte 1970). Immunocytochemistry is considered the most reliable method to identify C cells (Kameda et al. 1988), and these cells can be further classified into two subtypes (Robertson 1986). It is still uncertain to what extent the "parenchymal" and "vesicular" components of the gland are related to endocrine and non-endocrine functions and how they interact with each other.

8.3.6.2 Function

Calcitonin helps regulate calcium and phosphorus levels in the body. Thus, the hormones parathormone and calcitonin of the UB must be in balance if the calcium levels in the blood are to be in balance to requirements. The secretion of calcitonin by the UB is stimulated by high levels of calcium in the blood. Calcitonin acts to reduce blood calcium levels by inhibiting bone resorption and increasing the excretion of calcium by the kidneys (McConn et al. 2019).

The UB interacts closely with the thyroid gland to regulate thyroid hormones' synthesis and calcium level. Calcitonin produced by the UB acts as a negative feedback mechanism, inhibiting the release of thyroid hormones from the thyroid gland (Ueck 1979).

The UB's role in calcium homeostasis and bone development has implications for poultry management, particularly in optimizing skeletal health and eggshell quality in chickens. Manipulating UB function and calcitonin secretion may contribute to the prevention and management of calcium-related disorders in poultry production. Environmental factors, including light cycles and nutritional status, can influence UB function and calcitonin secretion in birds. Photoperiod and dietary calcium levels impact UB activity and calcitonin production (McConn et al. 2019).

8.3.7 Carotid Body

A relatively small glomus cells close to the common carotid artery surrounded by connective tissue capsule are observed in chicken (Figure 8.6). It has been reported that glomus cells may be present around branches off the common carotid artery or embedded inside the parathyroid gland (Kameda 1990).

8.3.8 Pineal Gland (Epiphysis Cerebri)

The pineal gland (pineal body) is a small gland that uses tryptophane to produce melatonin. It develops from the roof of diencephalon during embryonic development.

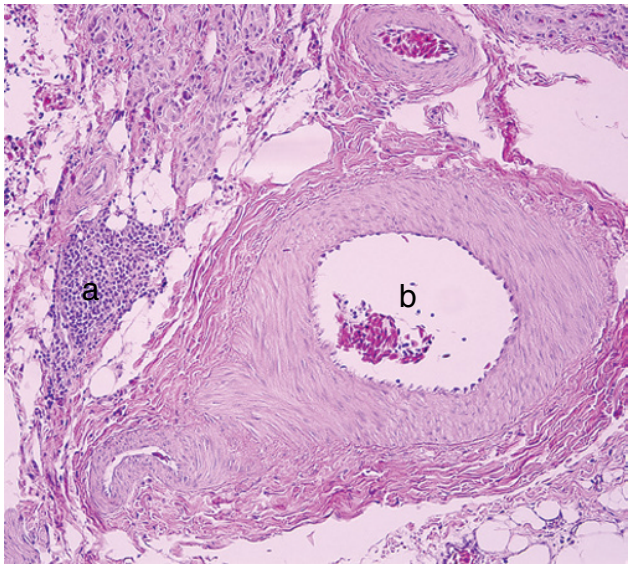

Figure 8.6 Carotid body of chicken. (a) Aggregates of carotid body (chemosensory) cells, close to (b) common carotid artery. H&E stain.

Melatonin affects sleep, behavior, and brain electrical activity. Thus, the pineal body acts as a biological clock that regulates the circadian rhythms and the sleep-wake cycles and, as such, influences the activities of the hypothalamus and its production of releasing hormones.

8.3.8.1 Anatomy and Histology

The avian pineal gland is a small endocrine organ located above the mid-brain, between both cerebral hemispheres, and its characteristics can vary significantly among different species (Quay and Renzoni 1967; Binkley et al. 1989). It is often situated near the surface of the brain and is richly vascularized. In some species of birds, such as chickens and turkeys, the pineal gland may be relatively large and elongated, while in other species of birds, it may be smaller and more rounded.

It consists of pinealocytes, which are the primary secretory cells responsible for the synthesis and release of melatonin, along with other cell types, such as glial cells and interstitial cells (Binkley et al. 1989). The primary cell types found in the pineal gland are modified photoreceptor cells. The pineal gland acts as a biological clock and influences the activities of the hypothalamus. The pineal gland also contains supportive elements associated with glial cells or ependymal cells, as well as intrinsic neurons that project through the pineal tract to hypothalamic regions (Korf et al. 1982). The intrinsic neurons are typically concentrated in the proximal region, known as the stalk, of the pineal gland. The avian pineal gland receives sympathetic innervation from the superior cervical ganglion; however, the extent and characteristics of this innervation can differ significantly among bird species (Ueck 1979).

8.3.8.2 Function

The pineal gland in birds is involved in the regulation of circadian rhythms and the production of melatonin, a hormone with diverse physiological functions. Melatonin is synthesized and released in a rhythmic pattern, primarily during the dark phase of the light-dark cycle and plays a crucial role in coordinating various physiological processes, including sleep-wake cycles, reproduction, and immune function (Saad et al. 2023).

The pineal gland and its melatonin production are essential for photoperiodic responses and seasonal adaptations in birds. Changes in day length and light intensity influence the duration of melatonin secretion, which in turn triggers physiological and behavioral responses, such as molt, migration, and reproduction (Underwood et al. 2001). The pineal gland in birds is also thought to play a role in the regulation of seasonal behaviors, such as migration and breeding.

The pineal gland is regulated by photic input received through the eyes. Light information is transmitted via the retinohypothalamic pathway to the SCN of the hypothalamus, which, in turn, sends signals to the pineal gland to modulate melatonin synthesis and secretion (Cassone 1990). Manipulating light exposure and photoperiod can be used to regulate reproductive cycles, induce molt, and optimize growth and production parameters in poultry (Yalçın et al. 2018).

8.3.9 Islets of Langerhans

The islets of Langerhans are critical endocrine structures located within the exocrine pancreas of vertebrate animals, including chickens, responsible for the production and secretion of various hormones involved in glucose homeostasis (Ku et al., 2000).

8.3.9.1 Anatomy and Histology

The islets of Langerhans are small clumps of special cells located within the exocrine portion of the pancreas, which sit in the duodenal loop. These special cells produce two hormones: insulin, which lowers blood sugar, and glucagon, which thus increases blood sugar and affects fatty acid levels.

The chicken pancreas consists of three main pancreatic lobes (splenic, dorsal, and ventral) plus an additional lobe called the "third lobe" (Ruffier et al. 1998). In birds, including chickens, the islets of Langerhans are distributed throughout the pancreas (Figure 8.7A and B). They consist of several cell types, including beta, alpha, gamma, and pancreatic polypeptide (PP) cells (Dunning et al. 2005). Beta cells produce insulin, alpha secrete glucagon, gamma produce somatostatin, and PP cells synthesize PP.

Unlike mammals, the avian endocrine pancreas is characterized by two basic islet types: glucagon or A-islets, composed of many glucagon-secreting alpha-cells and a few somatostatin-secreting gamma-cells with occasional clumps of insulin-secreting beta-cells, and insulin or B-islets composed mainly of beta-cells with small numbers of gamma- and alpha-cells. The latter are often absent in this type of islets (Falkmer and Patent 1972; Bonner-Weir and Weir 1979; Ruffier et al. 1998). A third islet type they named mixed type or mammalian type islets composed of numerous beta-cells and only a few alpha- and gamma-cells. The A-islets and mammalian type islets are confined almost exclusively to the splenic and third lobes, whereas B-islets are distributed throughout all lobes (Bonner-Weir and Weir 1979).

8.3.9.2 Function

The islets of Langerhans play a central role in maintaining glucose homeostasis in birds. Insulin, secreted by beta-cells, regulates glucose uptake and utilization and promotes its storage as glycogen in the liver and muscle (Yin et al. 2016). Glucagon, produced by alpha-cells, increases blood glucose levels by promoting glycogen breakdown in the liver. Somatostatin, released by gamma-cells, regulates the secretion of insulin and glucagon and maintains glucose balance.

The islets of Langerhans respond to changes in blood glucose levels and other physiological signals. Glucose-stimulated insulin secretion from beta-cells is a critical mechanism for glucose homeostasis in chickens (Richards et al. 2010). Additionally, the control of glucagon secretion by alpha-cells contributes to the regulation of blood glucose levels. The islets of Langerhans in birds, including chickens, interact with gut hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide. These hormones enhance insulin secretion and promote satiety, regulating glucose metabolism and food intake (Yin et al. 2016).

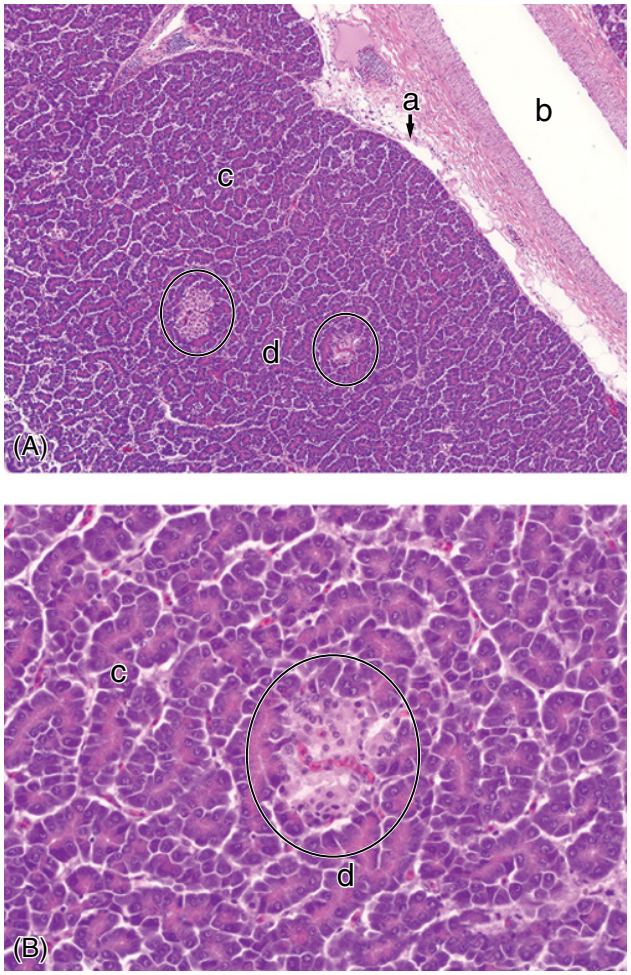

Figure 8.7 (A, B) Chicken pancreas showing (a) thin connective tissue capsule, (b) adjacent muscular artery, (c) exocrine pancreas, and (d) endocrine pancreas (small and large islets, encircled). H&H stain.

Environmental factors, such as temperature and photoperiod, as well as nutritional status, can influence the function of the islets of Langerhans in birds. Temperature fluctuations and photoperiod changes can affect insulin secretion and glucose metabolism, highlighting the interplay between the endocrine system and environmental cues (Yin et al. 2016). Nutritional imbalances and dietary composition also impact the function of the islets and overall metabolic health in chickens.

8.3.10 Gonads

The gonads are essential reproductive organs responsible to produce gametes and synthesis of sex hormones. The male gonads produce androgens (testosterone) which are responsible for the development and maintenance of male secondary sex characteristics. In female chickens, the ovaries produce estrogen and progesterone which are responsible for the development and maintenance of female secondary sex characteristics and behavior. These hormones also play a role in the maintenance of bone density and the regulation of metabolism. When a male is castrated, the balance of the sex hormones is affected which leads to the bird taking on female characteristics. This means that a capon, or castrated male, will over time take on much of the appearance and behavior of a female.

8.3.10.1 Anatomy and Histology

During embryonic development, the gonads differentiate from the indifferent gonadal primordia, with genetic and hormonal factors influencing their sexual differentiation (Guioli and Lovell-Badge 2007). In chickens, the gonads are located within the abdominal cavity near the kidneys.

The histological features of chicken testes include seminiferous tubules, which are lined with germinal epithelium, and comprise spermatogenic cells at various stages of development. Spermatogenesis, the process of sperm production, occurs within these tubules, Sertoli cells, which are supportive cells located within the seminiferous tubules and provide nutrition and structural support to developing germ cells. Leydig cells (interstitial) found in the interstitial spaces between the seminiferous tubules that produce testosterone. The testes are highly vascularized to provide oxygen and nutrients for spermatogenesis.

The histological features of chicken ovaries include ovarian follicles that consist of an oocyte (immature egg) surrounded by follicular cells that undergo growth and development, leading to ovulation. The granulosa cells are the main type of follicular cells surrounding the oocyte.

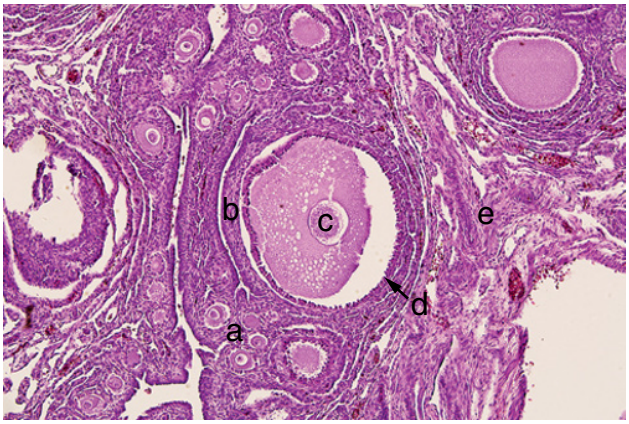

Figure 8.8 Histological section of a chicken ovary showing: (a) developing follicles at different stages of development, (b) mature follicle, (c) nucleus, (d) theca layers, and (e) ovary parenchyma. H&E stain.

They provide nourishment to the oocyte and secrete hormones like estrogen. Theca cells are found outside the granulosa cell layer, separated by the basement membrane, and are involved in hormone production, including the synthesis of androgens (Figure 8.8).

8.3.10.2 Function

Gonadal development and function in birds, including chickens, can be influenced by environmental factors and hormonal regulation. Photoperiod and temperature play significant roles in gonadal development, reproductive behaviors, and the regulation of seasonal reproduction (Fusani 2008).

The gonads are responsible for the process of gametogenesis, which involves the production of sperm in males and eggs (ova) in females. In the testes, spermatogenesis occurs, leading to the formation of mature spermatozoa (Alavi and Cosson 2006). In the ovaries, oogenesis takes place, resulting in the development and release of ova.

8.3.11 Digestive Enterochromaffin Cells

Enterochromaffin cells are the first endocrine cell type detected in the avian gut; subsequently, several types of such cells are distinguished based on their ultrastructural features of the secretory granules. Most endocrine cells are present in the proventriculus and small intestine but few in the gizzard, cecum, and rectum.

Experimental evidence has shown that most gut endocrine cells are of endodermal origin and are not derived from the neural crest or neuroectoderm as earlier proposed. In early embryos, the progenitors of gastrointestinal endocrine cells are more widespread than the differentiated cells in chicks at hatching (Rawdon 1984).

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9

Sense Organs

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9.1 The Eye

The purpose of vision is to enable the animal to forage, drink, communicate, hunt (insects and lizards), and escape danger. There are many differences between the chicken eye and that of mammals. The first includes a larger eye in chickens with a scleral cartilaginous or bony ring around the cornea. The bird's eye is large compared to the entire skull. The bony boundary of the two orbits is separated by a thin layer of bony plate with a big opening for communication between the two sides (Figure 9.1). The chicken is a diurnal animal (awake during the day) which means the eyes function in brightly lit environments. Like all diurnal birds, chicken exhibits a larger axial length of the eye relative to the corneal diameter (Hall and Ross 2007). Bony orbit shape in birds is influenced by their activity and vision physiology, and the development of the orbit is affected by the structure, amount, and organization of the surrounding soft tissue.

Bony orbit in birds does not have fat pads like that of mammals. The boundary of the bony orbit is the frontal bone dorsally, pterygoid/palatine ventrally, interorbital septum medially, lacrimal rostrally, and sphenoid/zygomatic caudally (Figure 9.1). Chicken eye fills the bony orbit with a short optic nerve, resulting in extremely limited movement. This limitation is compensated by the more movable head (atlanto-occipital joint) and the long movable neck (Getty 1975, p. 2066).

9.1.1 Eyeball

The eyeball or globe has three tunics from outside in they are fibrous, vascular, and nervous.

9.1.1.1 Fibrous Tunic

The fibrous tunic consists of the sclera and the cornea.

9.1.1.1.1 Sclera The sclera is dense connective tissue, consisting of collagen fibers that form a cartilaginous structure known as the sclerotic ring. The sclerotic ring, initially composed of hyaline cartilage undergoes ossification with age. Additional bone near the optic nerve exit site is present in certain bird species, eventually taking on a crescent-shaped bone structure. This optic bone (*os opticum*) is absent in chicken (Koch 1973, p. 142). Eighty percent of the scleral bony rings contain an average of 14 bones. During development, each bone is induced by a transient papilliform thickening in the overlying conjunctival epithelium. These conjunctival papillae appear on the 8th day of incubation and disappear on the 12th day, when the corresponding pre-osseous membranes begin to ossify (Coulombre and Coulombre 1973).

Because of the scleral bone attachment to the scleral soft tissue, it appears completely separated when one looks at the skeleton of a bird. This cartilaginous bony ring is for support and protection of the soft internal parts of the eye.

The sclera consists of an outer fibrous and an inner cartilaginous layer. The fibrous part consists of tightly packed collagen bundles, a few elastic fibers, and flattened fibroblasts in all the age groups studied. The presence of densely packed collagen bundles may resist the intraocular pressure and prevent the eyeball from changing its shape. It also serves as an insertion site for the extraocular muscles. The deep layer of the sclera continues with the pia mater that covers the optic nerve. This relationship results in the formation of the lamina cribrosa (perforated discs) where the optic nerve axons leave the eye in chickens and in mammals (Eurell and Frappier 2006, p. 351).

Anatomy and Histology of the Domestic Chicken, First Edition. Edited by Wael Khamas and Josep Rutllant.

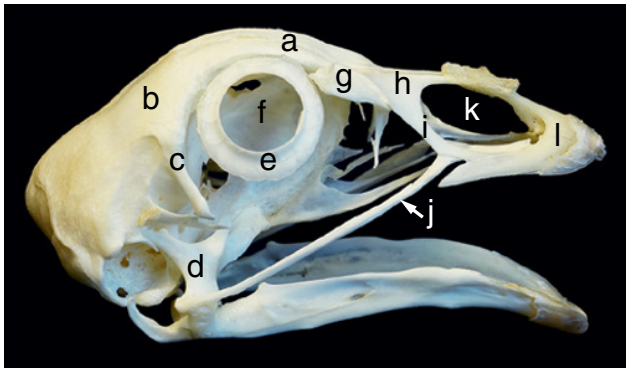

Figure 9.1 Chicken skull showing bones including scleral ossicle and structures close to the orbit. (a) Frontal bone, (b) parietal bone, (c) postorbital process, (d) quadrojugal, (e) scleral ring, (f) orbital cavity (bony orbit), (g) lacrimal (prefrontal) bone, (h) nasal bone, (i) lateral ramus of the nasal bone, (j) maxilla, (k) external nares opening, and (l) premaxilla.

9.1.1.1.2 Cornea The cornea is the most anterior layer of the fibrous tunic which allows the light to pass through into the eye. It ends in a groove-like structure at the junction with the sclera called the limbus. It is also attached to the internal border of the sclerotic ossicle. The anterior surface of the cornea is covered with stratified squamous epithelium and simple cuboidal epithelium covers the posterior surface which is in contact with the anterior chamber of the eye (Figures 9.2 and 9.3). The corneal epithelium rests on a basement membrane (Bowman's membrane) while the posterior limiting membrane is called Descemet's membrane. The cornea is the transparent portion of the fibrous tunic which allows the light to pass through to reach the retina. It is kept moist by the secretion of the lacrimal, and Harderian glands (Jones et al. 2007). The cornea is the most anterior part of the eyeball while the sclera forms the posterior part.

9.1.1.2 Vascular Tunic (Uvea)

The vascular tunic of the eye, also referred to as the uvea, is positioned beneath the sclera and closely adheres to it. This tunic is composed of three distinct regions, namely the choroid, ciliary body, and iris, listed in a posterior-to-anterior sequence. In addition, there is a unique structure called the pecten.

9.1.1.2.1 Choroid The *choroid* is a thin, variably pigmented, vascular tissue forming the posterior uvea. It joins the ciliary body anteriorly and lies between the retina and sclera posteriorly. The choroid is extremely vascular, with its capillaries arranged in a single layer on the inner surface to nourish the outer retinal layers (Miller 2009).

The choroid comprises blood vessels, melanocytes, fibroblasts, resident immunocompetent cells, and supporting collagenous and elastic connective tissue. It supplies oxygen and nutrients to the outer retina, and, in species with avascular retinas, to the inner retina as well. Other likely functions include light absorption (in species with pigmented choroids), thermoregulation via heat dissipation, and modulation of intraocular pressure via vasomotor control of blood flow. The choroid also plays an important role in the drainage of the aqueous humor from the anterior chamber, via the uveoscleral pathway (Nickla and Wallman 2010).

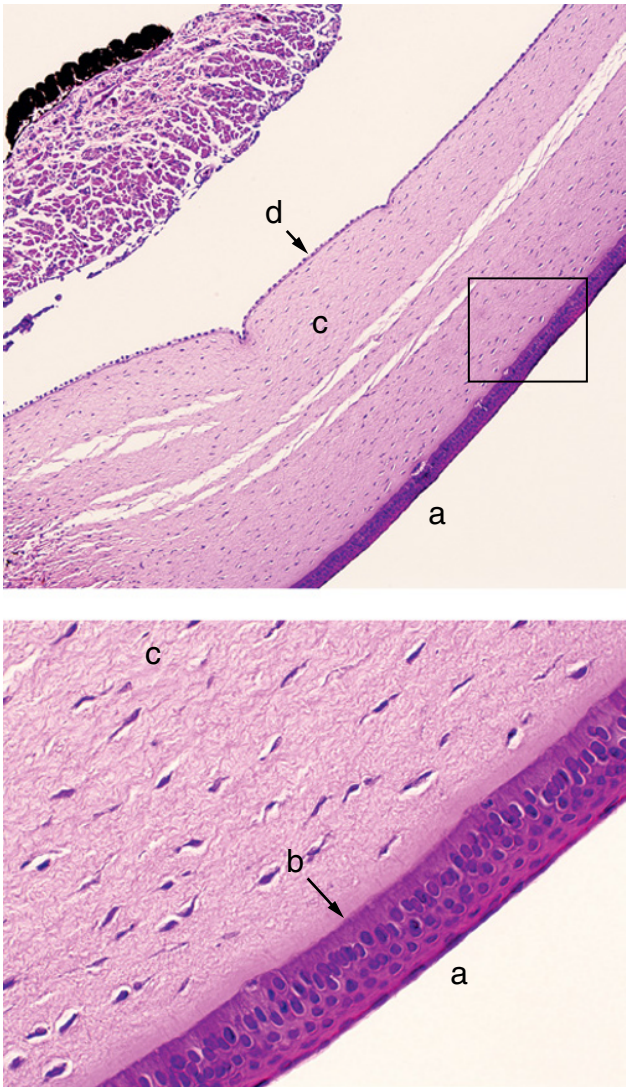

Figure 9.2 Histological section of the chicken cornea showing (a) anterior non-keratinized stratified squamous epithelium, (b) Bowman's membrane (external limiting lamina), (c) proper substance of the cornea with connective tissue and fibrocytes, and (d) posterior corneal epithelium (simple cuboidal). The Descemet's (internal limiting lamina) membrane is not clear in the images. H&E stain.

9.1.1.2.2 Ciliary Body The ciliary body lies immediately posterior to the iris. On its posterior surface, the ciliary body has numerous folds known as the ciliary processes.

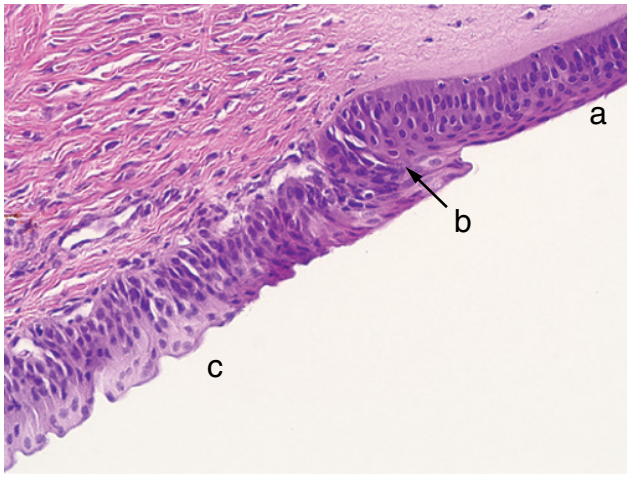

Figure 9.3 Corneal conjunctival junction, (a) cornea with non-keratinized stratified squamous epithelium, (b) line of transition into the bulbar conjunctiva showing the transition between stratified squamous (a) and the pseudostratified columnar epithelium (c). H&E stain.

This area of the ciliary body, termed the *pars plicata* (folded part), merges posteriorly into a flat area (pars plana), which joins the retina. The zonular fibers, which support the lens, originate from the pars plana and between the ciliary processes.

The ciliary processes in chicken are like those of mammals. The only difference reported is the branching of the processes posteriorly forming ridges (Smith and Raviola 1983). The ciliary body fixes the lens with short zonule fibers because it has a direct attachment to the lens capsule which is different than mammals. Through this kind of attachment, it becomes more effective in changing the shape of the lens and the cornea for better vision (accommodation). The presence of the radial striated (skeletal) muscle forces the ciliary body against the lens so that the curvature of the lens surface is increased (Getty 1975, p. 2064). This muscle is supplied by motor neurons carried within the oculomotor nerve. The ciliary processes attach to the lens and help in regulation of the aqueous humor fluid through the venous sinuses (ciliosclera sinuses) situated at the angle between the scleral corneal junction. These delicate connective tissue fibers called reticulum are where aqueous humor drains into the scleral venous sinuses.

The ciliary body with its processes alters the focal power of the lens, produces aqueous humor that supplies nutrition to ocular structures, and aids in regulating intraocular pressure. Together they also form a blood-aqueous barrier to maintain the clarity of the aqueous humor and vitreous body. The choroid plays a major role in providing nutrition to the retina. In chickens as in mammals' tight junctions between the non-pigmented cells of the ciliary epithelium represent the structural equivalent of the blood-aqueous barrier (Smith and Raviola 1983).

The ciliary body is covered with two layers of epithelium, the inner layer of which is non-pigmented and the outer layer is pigmented. It is continuous with similar epithelium on the posterior surface of the iris and the pigment epithelium of the retina (Figures 9.4 and 9.5).

9.1.1.2.3 Iris The chicken iris is yellow brown in color and surrounds a round pupil which has a constrictor and dilator striated muscles to control the amount of light entering the eye. In chicken, the iris is unresponsive to light. No tapetum lucidum is described in chicken (Dyce et al. 2010, p. 813).

The iris forms a complete ring around the pupil (the opening that allows the light to pass through). The iris has anterior and posterior pigmented simple cuboidal epithelium. In between the two epithelial layers, there are the

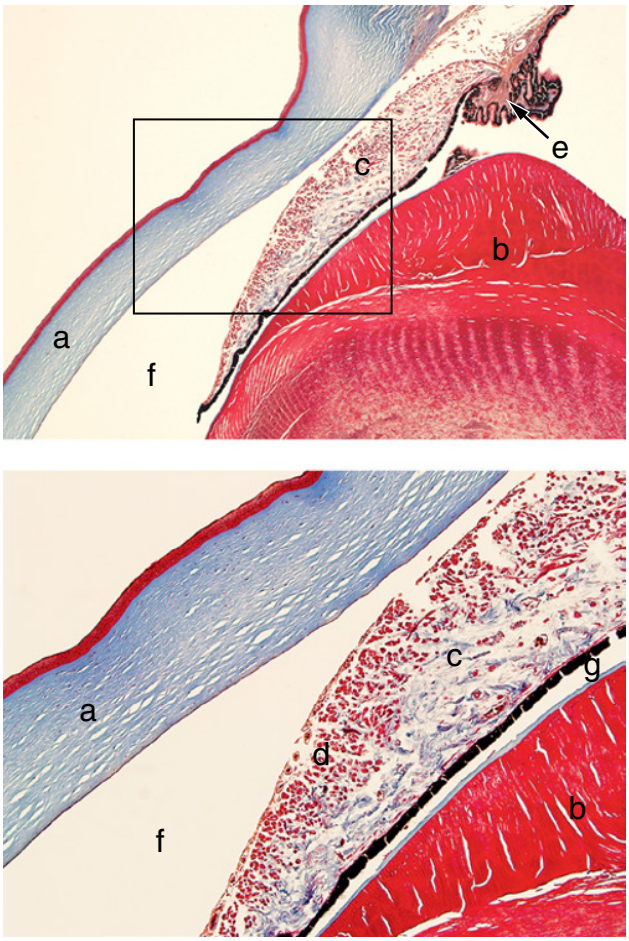

Figure 9.4 A section through the eyeball showing the (a) cornea, (b) lens, (c) iris, (d) iridial skeletal muscle, (e) ciliary processes, (f) anterior chamber, and (g) posterior chamber. Trichrome stain.

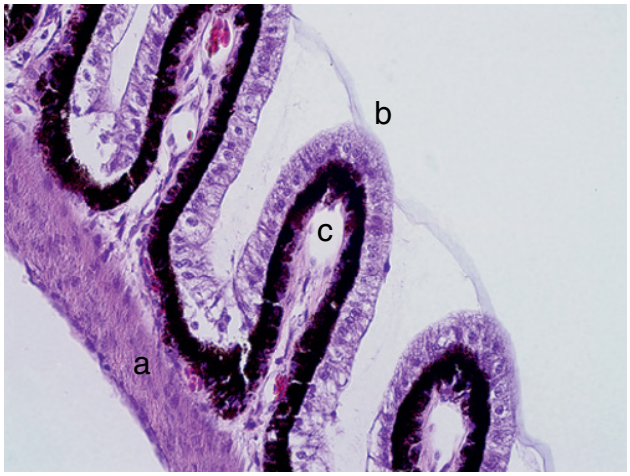

Figure 9.5 Ciliary processes arise from the (a) pars plicata (connective tissue base), (b) ciliary folds (plicae ciliaris) line with non-pigmented simple cuboidal epithelium, (c) outer pigmented epithelium. H&E stain.

pigmented stroma and the circular and longitudinal skeletal muscles. These voluntary muscles allow the bird to focus better and faster than mammals.

In the chicken eye, the iris muscle changes the curvature of the lens during accommodation, whereas the ciliary muscle is responsible for increasing the curvature of the cornea for corneal accommodation (Glasser et al. 1994, 1995).

The iris is anchored through its base to the sclera by the pectinate ligament. Neuroepithelial type of epithelium (*pars ciliaris retinae* covers the ciliary body) consists of an outer pigmented layer and inner non-pigmented simple columnar epithelium (Figure 9.4). It functions to supply the retina with nutrients and oxygen. Choroid thickness plays a role in moving the retina thus bringing the rods and cones into the plane of focus (Nickla and Wallman 2010).

9.1.1.2.4 Pecten The bird eye pecten is a unique structure that consists of folded blood vessels attached to each other by pigmented cells which adhere to the vitreous body. This structure forms one characteristic feature of the choroid. The pecten is a black trapezoid in shape measures 8-mm wide at the base and 5 mm at its free border (Seaman and Storm 1963). Many studies linked the function of the pecten with the acuity of avian vision by absorbing light, though the acceptable theory is that the pecten acts as a trophic structure to the big bird eye by supplying the considerable number of layers of the retina with needed oxygen. In addition, the pecten acts in the regulation of the intraocular pressure (Wingstrand and Munk 1965; Brach 1977; Jasinski 1973).

9.1.1.3 Nervous Tunic (Retina)

The third tunic is the retina. In birds, there is no fovea nor central artery of the retina. Therefore, the presence of pecten is essential to supply the retina with oxygen and nutrients. The area centralis has a high number of cones and ganglionic neurons.

The retina develops as an evaginated vesicle from the diencephalon that forms a cup at the early stages of embryonic development. This cup invaginates to form a double-layered retina. The inner layer forms the sensory layer of the retina (ganglionic layer) while the outer layer forms the pigmented layer of the retina. The pigmented layer is rigidly attached to the underlying layer of the choroid. The two are remarkably close to each other, though retinal detachment is possible between these two layers. The retina is divided into an anterior and posterior portion. The anterior portion, pars ceca retinae, is the non-sensory part of the retina. It covers the inner surface of the choroid and ciliary body. The posterior portion of the retina is the sensory portion. The separation between the two portions of the retina is called ora serrata (ciliary body and ciliary processes). The corresponding structure of the optic papilla in mammals is not obvious in chicken because it is covered completely by the pecten of the choroid tunic. The thickness of the retina varies among species and in chicken it has the regular 10 layers (internal limiting membrane, nerve fiber layer, ganglionic cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, external limiting membrane, outer nuclear layer, rods and cones, and pigmented layer of the retina). A chicken's retina contains rods, and double-cone photoreceptors that allow a chicken to see red, blue, and green, in addition to light frequencies on the ultraviolet spectrum (Lewis and Gous 2009). Ultraviolet light influences the pineal gland to release melatonin. The double-cone photoreceptor, a specialized receptor found in birds, provides sensitive motion detection and allows chickens to be adept hunters of small prey items. When given the opportunity, chickens are known to be efficient and ruthless predators of insects, lizards, mice, and snakes. (Kram et al. 2010). The researchers managed to detect five functional classes of cones using the colored oil droplets in the inner segment of the cone photoreceptors to characterize their special distribution in chicken eye. The optic nerves leave the eyeball, and all nerve fibers decussate at the optic chiasm. Therefore, consensual pupillary light reflex cannot be elucidated.

9.1.2 Lens

The avian lens, along with the cornea, serves to refract the light that enters the eye. In addition, the lens focuses light onto the surface of the retina, creating an acute and focused image. This is accomplished by its transparency, avascularity, and accommodative properties. The lens is held in place by the vitreous body and the zonular fibers that bind

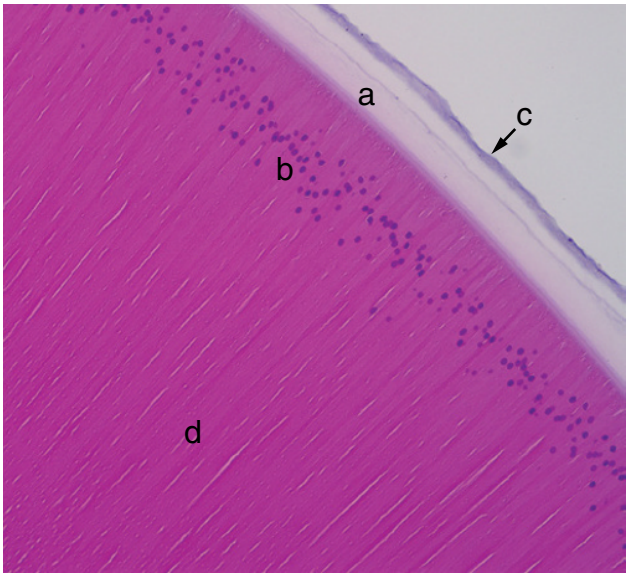

Figure 9.6 Histological section of the chicken lens showing (a) lens capsule, (b) simple cuboidal to columnar (toward the lens equator) cells covering most of the anterior portion of the lens except for the area where the lights pass through (pupil), (c) remnant of the zonular fibers, (d) lens fibers. H&E stain.

the ciliary process to the lens capsule. Unlike mammals, the ciliary muscles in chickens, both anterior and posterior, are skeletal in nature rather than smooth muscles. Oxygen and nutrients of the lens are provided by the aqueous humor. The lens is covered by simple cuboidal epithelium located below the capsule. The stroma of the chicken lens exhibits specific patterns of cellular nuclei covering the entire lens with specific arrangement at the pupil opening (Figure 9.6). Additionally, a notable feature of the chicken eye is the arrangement of radially oriented prisms encircling the central core of the lens, forming what is known as the annular pad. These prisms feature surface indentations corresponding to the number of ciliary processes they connect with (König et al. 2016, p. 229).

9.1.3 Eye Chambers

The eye has two chambers, an anterior chamber located between the cornea and the iris and a posterior chamber between the iris and the lens with the zonule fibers. Both chambers are filled with aqueous humor. The two chambers communicate through the pupil (Figure 9.4). The largest compartment of the eyeball is behind the lens occupied by the vitreous body.

The aqueous humor is present within the anterior and posterior chambers of the eye, and it is in contact with the cornea and the lens. Because both structures have limited blood supply, the aqueous humor supports the nutrient and oxygen to all structures in contact. The aqueous humor is secreted into the posterior chamber by the ciliary body epithelial cells (Trejo-Reveles et al. 2018). It passes through the pupil into the anterior chamber. The fluid can be regulated through a trabecular meshwork within the iridocorneal junction, where veins drain it to the general circulation. Intraocular pressure is maintained at a healthy level when production and drainages are regulated and in equilibrium (Fautsch and Johnson 2006). Any change in intraocular pressure will affect vision and the health of tissues within the eye.

9.1.4 Barriers

Blood barriers are scattered in many organs within the animal body, including the eye. The basis of most barriers is the presence of tight junctional complexes between adjacent cells. Other important barrier components are the modifications within the blood capillaries from discontinuous basal lamina to fenestrations in order to regulate the passage of large molecular weight substances. The first barrier in the eye is the blood-aqueous humor barrier. It has been demonstrated that chicken ciliary body blood vessels are composed of a thin endothelial lining and an incomplete thin basal lamina. Large molecules of horse radish peroxidase (HRP) were used to pinpoint the exact region where they may pass or stop because of the presence of certain structural modifications. Smith and Raviola (1983), using transmission electron microscopy, stated that HRP passed through the spaces between the non-pigmented and pigmented epithelial cells but failed to pass through the tight junctions on the lateral surface of the non-pigmented epithelial cells.

9.1.5 Vitreous Body

The vitreous body occupies the posterior compartment of the eye globe. It is composed mostly of water and hyaluronic acid laid on a collagen base. Other components include glucose, ions, and proteoglycans, which make it a gel-like consistency. It is surrounded by an extremely thin membrane called hyaloid membrane (vitreous membrane) (Baumel 1993) composed of collagen fibers. This membrane attaches to the ora serrata as well as the posterior part of the retina. The latter is prone to detachment from the retina. The vitreous body maintains the eyeball shape, allows the light to pass through to reach the sensitive layer of the retina, and supports the retina by keeping it and the lens in place (Wright and Mayne 1988).

9.1.6 Adnexa

9.1.6.1 Eyelids (Palpebra; Plural Palpebrae)

The bird has superior and inferior eyelids (palpebrae) (Figure 9.7). The superior eyelid, also called palpebra

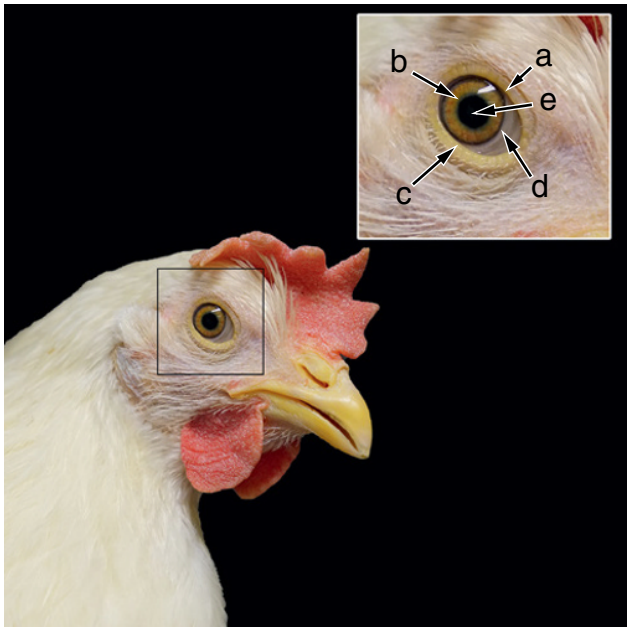

Figure 9.7 Female chicken head showing (a) upper eyelid, (b) iris, (c) lower eyelid, (d) third eyelid (nictitating membrane), and (e) pupil.

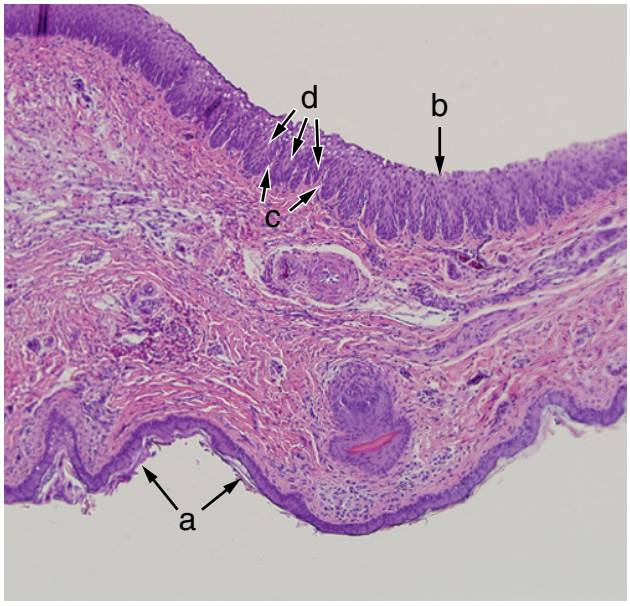

Figure 9.8 Upper eyelid of the chicken showing: (a) relatively thin stratified squamous epithelium with a thin layer of keratin on the external surface, while (b) the internal surface (palpebral conjunctiva) has thicker non-keratinized stratified squamous epithelium with (c) dermal papillae and epidermal pegs (d). H&E stain.

frontalis, is usually short and less movable. Therefore, it does not contribute to the palpebral reflex (blinking). The inferior palpebra is called palpebra maxillaris which is longer than the superior and more movable (Koch 1973, p. 142). Feathers of small sizes attach to the superior eyelid and function like the eyelashes in mammals. In both eyelids, near the edges, there are modified eyelashes called filoplumes, that serve as protection and tactile function. The superior (upper) eyelid is raised by the levator palpebral superior muscle, and the inferior eyelid is depressed by the depressor palpebral inferior muscle. The eye's opening and closing movements are controlled by the combination of these two muscles which are innervated by the trigeminal nerve along with the orbicularis oculi muscle located on the free edges of the eyelids which is innervated by the facial nerve. Externally, both eyelids have stratified squamous epithelium with the upper one having higher stratification (Figure 9.8). No glands were detected at the edges of either eyelid. The external part of the superior palpebra is lined with a thin layer of stratified squamous epithelium, while the inner layer of the superior palpebra (palpebral conjunctiva) is lined with a thick stratified cuboidal epithelium (Fix and Arp 1991). This epithelium interdigitates with the underlying tissue through the epidermal pegs. Balloon-like cells observed in the middle of the eyelid indicate a transitional nature of the epithelium. The eyelid is highly vascularized and innervated (Figure 9.8). Dendritic cells are observed along with intraepithelial lymphocytes. Dendritic cells move through the tissue to detect antigens. These cells appear lightly stained with hematoxylin and eosin. These cells could be positioned anywhere within the epithelium and are not to be confused with melanocyte and Merkel cells which are located within the *stratum basale* layer only.

9.1.6.2 Third Eyelid

The third eyelid (nictitating membrane) is a thin membranous structure attached to the medial canthus of the eye and free on its lateral aspect (Figure 9.9A and B). It covers the entire eye when fully extended. It is used to clean, moisten, and protect the cornea by making extremely rapid excursions over the surface (Schobert et al. 2013). The external surface of the third eyelid is covered by non-keratinized stratified squamous epithelium. Histologically, it is like the outer layer of the eyelids, but the epithelium is thinner. This epithelium transitions to simple columnar and pseudostratified columnar epithelia with goblet cells toward the internal surface in contact with the lens (Figure 9.9). The third eyelid acts to protect the eye (fibrous layer, cornea) from any irritant including air. It also acts to spread the film tears over the fibrous tunic of the eye. Two muscles are described to act on the third eyelid, *musculus pyramidales palpebrae tertiae* and *musculus quadratus palpebrae tertiae*. These muscles are supplied by the abducent nerve (cranial nerve VI). The accessory gland of the third eyelid is described to be

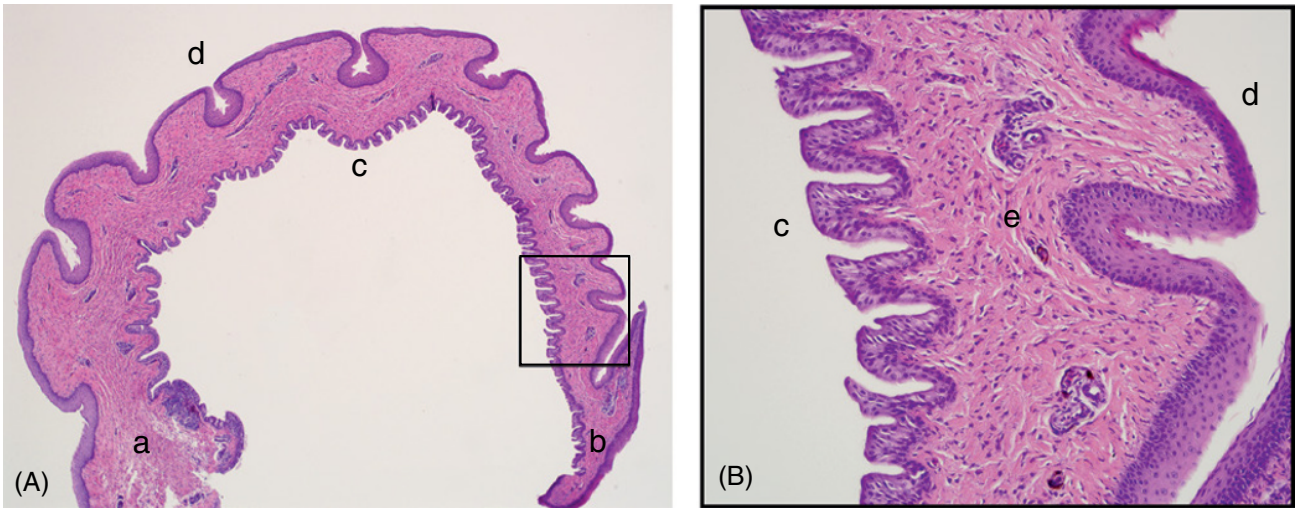

Figure 9.9 The third eyelid of the chicken (a) attached and (b) free ends. The free end of the third eyelid, showing (c) inner folded simple columnar to pseudostratified columnar epithelium and (d) less folded external surface covered with stratified squamous epithelium, (e) dermis (dense irregular connective tissue). (A) Low magnification showing the entire third eyelid, (B) Higher magnification showing the differences in the type of epithelium showing lightly stained goblet cells within the inner epithelium (c). H&E stain.

loosely attached to the periorbita and will be described in more detail later in this chapter. The periorbita is a connective tissue sheath that surrounds the entire eyeball and its adnexed structures.

9.1.6.3 Extraocular Muscles

All eye muscles are extremely thin because of the limited motion of the eyeball in birds. The retractor bulbi muscle is absent in chickens and replaced by the quadratus and pyramidalis muscles. Similar to mammalian, the external ocular muscles in birds consist of four recti muscles named superior (dorsal), inferior (ventral), medial, and lateral, and two oblique muscles (superior and inferior).

9.1.6.4 Lacrimal Apparatus

9.1.6.4.1 Lacrimal Gland The lacrimal gland is located medial to the caudal surface of the lower eyelid, and it is smaller than the accessory lacrimal gland (Harderian). Its lining epithelium varies from simple cuboidal, simple columnar, or stratified with goblet cells. In the fowl, a single duct leaves the posterior extremity of the lacrimal gland to open onto the deep surface of the lower eyelid.

9.1.6.4.2 Harderian Gland (Third Eyelid Gland) The third eyelid gland is within the bony orbit, loosely attached to the outside of the periorbital fascia, and larger than the lacrimal gland (Wight et al. 1971). It has a single duct that opens into the conjunctival sac at the base of the nictitating membrane. The ducts of the Harderian and lacrimal glands were lined by a single layer of epithelium, the cells of which varied from cuboidal to columnar, though stratified, and pseudostratified regions were also seen (Burns and Maxwell 1979). Histologically, the Harderian gland of the domestic fowl is classified as compound tubulo-acinar, pale pink to brownish red and irregular in shape and strap-like. The Harderian gland of the fowl lies in the orbit ventral and postero-medial to the eyeball. It extends rostrally from the region of the optic nerve. Its duct emerges from its anterior extremity. If the eye is removed the gland will remain within the orbital cavity. The apical portion of the glandular part is darkly stained and serous in nature while the basal portion is lightly stained and mucous in nature (Figure 9.10). A number of plasma cells, varied sizes of lymphocytes, mast cells, macrophages, and melanin pigments were present in the interlobular tissue of the gland (Baba et al. 1990).

The acini are lined by variable heights of columnar epithelium depending on their functional status. The basally situated nucleus is circular and a basophilic nucleolus is usually visible (Wight et al. 1971).

It is also stated that the size of the Harderian gland varied between the broiler and native boiler chickens of Bangladesh. The length and breadth of the Harderian gland in the male broiler and native chickens were found to be higher in comparison to the female chickens. The plasma cells and lymphocytes were in the apical part of these acini and in the inter-acinus spaces (interstitium) of the broiler and native chicken (Jahan et al. 2006).

The Harderian gland is populated by many plasma cells and linked to the bursa of Fabricius in its development (Mueller et al. 1971). Therefore, they concluded that this

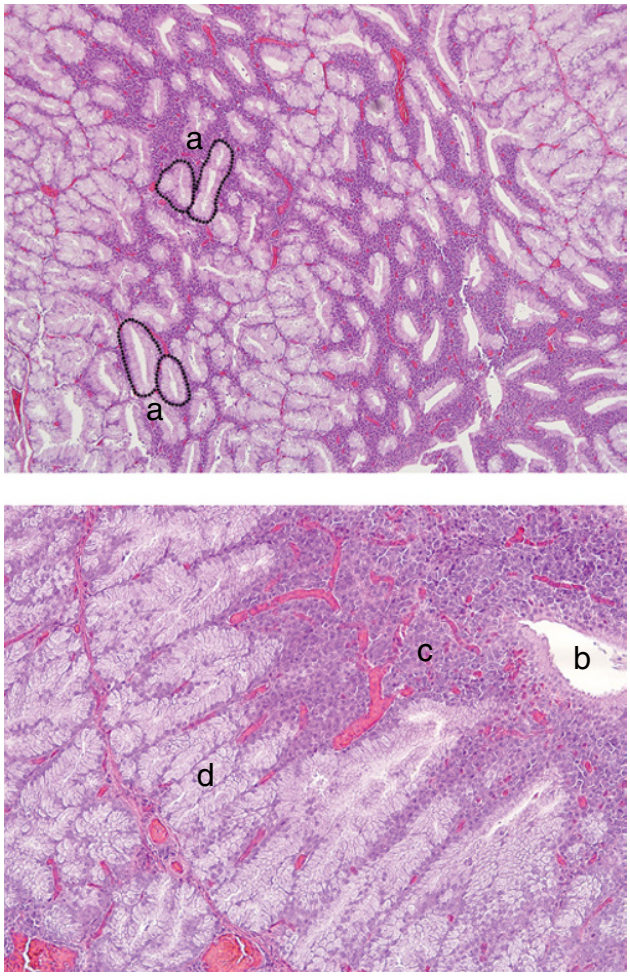

Figure 9.10 Harderian gland of the chicken (lacrimal gland of the third eyelid), (a) acini are within the lobes with (b) centrally located duct collecting the secretory materials, (c) apical darkly stained cells due to lymphocytic infiltration and the rest of the gland lobule are (d) lightly stained. H&E stain.

gland may contribute to the immune system of the chicken, especially with the presence of well-organized lymphoid follicles with germinal centers. The Harderian gland gets its blood supply from the external ophthalmic artery and innervation through a ventral branch of the third cranial nerve, the oculomotor.

9.1.7 Vision

The change in corneal curvature results in changing the refractive index. Therefore, muscle movement will affect the corneal curvature and the zonule fibers to change the lens curvature. The close relation of the scleral ossicle with the cornea and the ciliary muscles allows the structures to withstand the pressure caused by the corneal accommodation (Fischer and Schoenemann 2019).

9.2 The Ear

Like mammals, the chicken ear is composed of three compartments: the external ear, the middle ear, and the internal ear. The external pinna is absent, and the external ear has only an orifice surrounded by very tiny feathers. The feathers protect the external ear canal from ectoparasites or flies, allowing sound to go through. The columella is a thin bony plate attached to the tympanic membrane, equivalent to the stapes in mammals, which transmits the sound waves into the inner ear's perilymph. Besides, the tympanic membrane in birds is uniquely connected to the quadrate bone which allows for transmission of sounds. The inner ear of chickens contains both the osseous (bony) and membranous labyrinths, functioning similarly to that of mammals, but with the notable difference that the chicken ear tends to be more sensitive to lower frequency sounds than that of mammals. Chicks develop audition by day 12 of incubation and by day 19 they start using clicking sounds to communicate with other chicks in the clutch and the hen to synchronize hatching. External influences, such as vocal-auditory interactions between the hen and chick, between chicks in the same clutch, or both, may influence the timing (Tong et al. 2013). This synchronization is a critical aspect of chick survival and allows the hen and the chicks to leave the nest sooner in search of food and water. Jones et al. (2006) conducted a study on chicken embryos to investigate their responses to sound and frequency selectivity. The research revealed that these responses began to be observed on day 15 of incubation. However, due to limited high-frequency sound transmission from the air to the cochlea during the late stage of chicken embryo development, their response to airborne sound remained constrained. Subsequently, there was a rapid maturation of frequency selectivity from day 16 to 18, resulting in a matured range of 170–4478 Hz.

9.2.1 Mature Chicken Ear Anatomy

Chicken ear consists of external, middle, and inner segments like mammals. The chicken ear is unique and differs from that of mammals in several aspects. It is embedded inside the temporal and the quadrate bones. One noticeable difference between birds and mammals is the linkage between the tympanic membrane and the quadrate bone. Additionally, avian auditory systems possess a solitary ossicle, known as the columella, in contrast to mammals, which have three ossicles. The columella comprises trifurcate cartilaginous extensions, referred to as extracolumella, which facilitate the connection between the tympanic membrane and the columella through multiple suspending ligaments. Notably, only one muscle, the

stapedial muscle, has been described in avian species (Muyshondt et al. 2017). The trifurcated extra-columella establishes a connection between the tympanic membrane and the columella. The central arm, known as the extra-stapedius portion, imparts a conical shape to the tympanic membrane, with the apex directed outward toward the ear canal. The quadrate bone plays a role in the beak suspension system, being linked to the tongue. It has been observed that sound attenuation differs between hens and roosters, utilizing both the quadrate bone and the beak opening for this purpose (Bock 1964). Additionally, the chicken's middle ear is interconnected with the contralateral ear through the pharyngotympanic tube and air spaces (Larsen et al. 2016).

The flattening of the membrane cone is attributed to a reduction in tension. When the eardrum's tension is relaxed, it leads to a decrease in sound transmission, implying that the extensive beak opening during a rooster's vocalization behavior influences the interaction with the quadrate bone. In hens, quadrate rotations lead to an increase in tension of the tympanic membrane. In roosters, a more pronounced rotation of the quadrate leads to a decrease in tension of the tympanic membrane (Muyshondt et al. 2017). Chickens tend to be more sensitive to lower frequency sounds, reflecting the significant role in low-frequency vocalizations in their social communication compared to other bird species and humans (Corfield et al. 2013).

The size of sound-producing structures can impose limits on the frequencies that songbirds can produce. Similarly, the size of the tympanic membrane and middle ear bone (columella in birds) can impose limits on the frequency range of hearing. However, the covariation between vocal frequency content and the range of high-frequency hearing can also be due to differences in body size (Greenewalt 1968; Henry et al. 2016).

9.2.1.1 External Ear

The external ear canal is an embryonic remnant of the first branchial cleft, and it is a simple epidermal invagination from the skin. Thus, it is lined with stratified squamous epithelium. Chicken has no external ear (pinna) but a small opening leads into the external acoustic meatus. The actual opening is only a few millimeters in diameter. It extends obliquely ventral and caudal of the external oval opening. This opening is circular and small, located caudal and ventral to the eye. The opening is covered by ear coverts, which are specialized contour feathers (Figure 9.11). These coverts lack barbules; therefore, they do not obstruct sound from passing through into the ear (Schmidt and Reavill 1996).

The external ear canal, like mammals, has ceruminous glands to secrete the cerumen. These glands secrete waxy material (cerumen) to function for the protection of the ear canal against physical damage and microbial infection. The external ear canal is separated by the tympanic membrane from the middle ear.

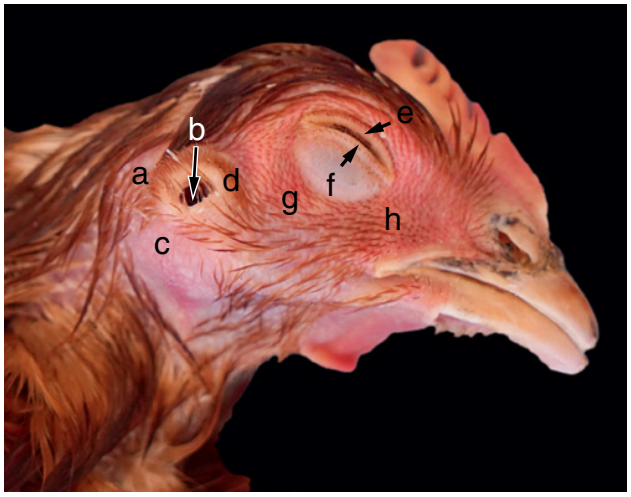

Figure 9.11 Lateral view of the head of a chicken showing the external ear opening and close-by regions. (a) Caudal ear covert, (b) external ear opening, (c) ear lobe, (d) rostral ear covert, (e) upper ocular tract, (f) lower ocular tract, (g) genal tract, and (h) malar tract.

9.2.1.2 Middle Ear

The single bone in the middle ear, the columella, is mesenchymal in origin which results from the condensation of cells within the second branchial arches (Cohen and Hersing 1993). The middle ear has two main portions, the pharyngotympanic tube (Eustachian tube) and the tympanic cavity. The tympanic cavity is an air-filled chamber that establishes a connection with the dorsal surface of the pharynx via a narrow slit-like opening known as the infundibulum. This opening functions to stabilize the pressure inside the middle ear and prevent rupture of the tympanic membrane under high-pitched noises. These functions are accomplished by the presence of several muscles including the stapedial and the tensor tympani muscles. The tensor tympani muscle originates from the occipital bone inserted on the medial surface of the tympanic membrane (Fujioka 1963). The connection of the middle ear with the pharynx allows air to enter the tympanic cavity for optimum function of the tympanic membrane. The sound enters through the small external ear opening and hits the large tympanic membrane, which transmits the sound into the perilymph of the inner ear. The columellar footplate inserts into the oval (vestibular) window at its proximal end. The presence of three processes (infra-columella, extra-columella, and supra-columella) positioned at a right angle to each other compensates for the absence of the

other two ear ossicles. The extra-columella attaches to the tympanic membrane at its distal end. The extra-columella has three processes. They support and contact the tympanic membrane. The tensor tympani muscle attaches to the infra-columella and the tympanic membrane.

The tympanic membrane in chicken is a curved conical shape which is opposite to that of mammals (Muyschondt and Dirchx 2020). During growth and further development of the head and general growth of the bird, the middle ear shows a remarkable change in size from neonatal to adult chicken (Saunders 1985; Claes et al. 2017). The tympanic membrane is covered from outside by stratified squamous epithelium like the external ear canal. The internal surface is lined by simple cuboidal epithelium which is continuous with the tympanic cavity and the pharyngotympanic tube. The tympanic membrane projects outward, opposite to mammals which projects inward. The cavity of the middle ear, the tympanic cavity below the membrane, is the conduit cavity between the external and the inner ear. The cavity opens into the pharynx via the pharyngotympanic tube (Eustachian tube) which connects the cavity to the oropharynx via the infundibular cleft. These connections between the different cavities, tympanic, and the oropharynx help in pressure equalization to prevent rupture of the tympanic membrane under high-pitched noises.

9.2.1.3 Inner Ear

The otic vesicle at early stage of embryonic development gives rise to the components of the inner ear, the labyrinth. The inner ear has the osseous (bony) and the membranous labyrinths. The bony labyrinth houses the perilymph while the membranous labyrinth has internally the endolymph. Both fluids are within the cochlea.

The osseous labyrinth includes the cochlea which functions for hearing. Lining the bony labyrinth is the membranous labyrinth of the utricle, saccule, and semicircular canals. These structures function to detect the balance and the movement of the head in space.

Bird bony cochlea is a short, slightly curved tube. It has three structures: the basilar papilla, the *tegmentum vasculosum* consisting of light and dark cells, and the otolithic macula at the distal tip. They are all lined by simple cuboidal epithelium. The macula has two sensory types of hair cells (tall and short) placed on a fibro-basilar membrane. The tall columnar cells are located over a fibrocartilaginous plate while the short cells have a surface diameter that is greater than height. They are situated at the free basilar membrane (Tanaka and Smith 1978). The cochlear duct filled with endolymph extends the entire length of the cochlear tube. Endolymph is rich in potassium ions. The fluid generates electrical (nerve) impulses from wave sounds. The perilymph is rich in sodium and chloride ions and transmits movements due to sound waves to the endolymph. Both endo- and perilymph are separated by the thin membranous labyrinth.

The regular three scalae (tympani, media, and vestibuli) are present in the chicken inner ear with a reduced scala vestibuli (Figure 9.12). Scala media and scala tympani are separated by a thick basement membrane. Endolymph flows inside both scala tympani and scala vestibuli (Getty 1975, p. 2068).

A slightly curved cochlea is present in chickens and is not spiral-like in mammals. The presence of sensory cells compensates for the differences with mammals. In mammals, sensory hair cells have extremely limited capability to divide and replace damaged cells. However, in chicken these cells have a better ability to do so through the presence of supporting cells which are quiescent and get activated when adjacent cells are damaged or died because of trauma (Stone and Cotanche 2007). The labyrinth contains several localized sensory areas of

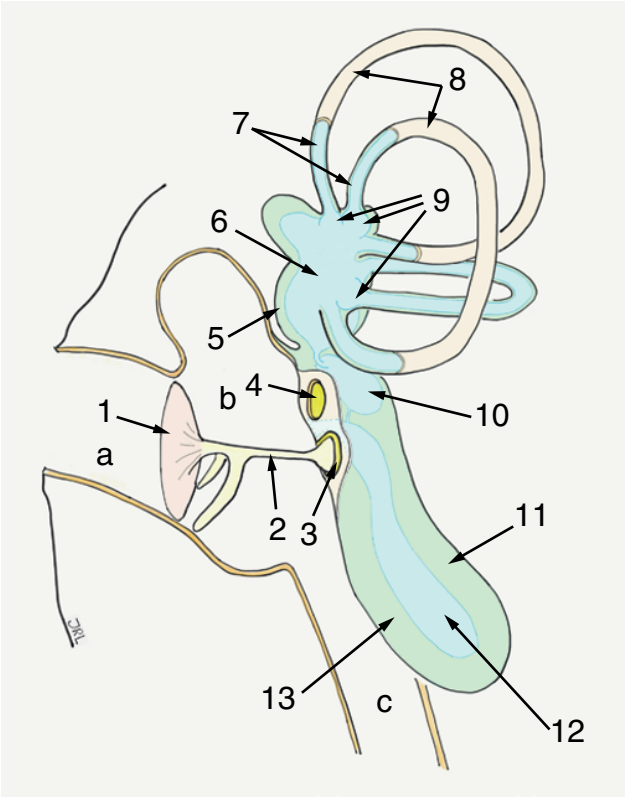

Figure 9.12 Diagram of the chicken ear. (a) External ear, (b) middle ear, tympanic cavity, (c) auditory or pharyngotympanic tube (connection from middle ear to the pharynx), (1) tympanic membrane, (2) columella, (3) oval (vestibular) window, (4) round (cochlear) window, (5) vestibule, (6) utricle, (7) semicircular ducts, (8) semicircular canal (osseus), (9) ampullae, (10) saccule, (11) scala tympani, (12) cochlear duct, and (13) scala vestibuli. The green fluid is perilymph, and the blue fluid is endolymph.

thickened epithelium (maculae, crests, and papillae) (Schmidt and Reavill 1996).

The hearing organ (basilar papilla) of the chick has a tubular form. The endolymphatic space is surrounded by a neuroepithelium (hair and supporting cells), specialized columnar cells connected with the tectorial membrane, and the tegmentum vasculosum (Hirokawa 1978). Intercellular junctions between hair cells and supporting cells exist. Junctional complexes (tight junctions and zonule adherents) join the hair cells to the processes of the supporting cells. A band of tight junctions seals the endolymphatic space, at the endolymphatic border, the hair cells are separated from one another by long processes of the supporting cells (Hirokawa 1980). The tectorial membrane of the avian ear is a thick and massive-appearing structure. It is not only in contact with the tips of the sensory hairs, but it is also attached to the supporting cells by means of fine fibrils. This is quite different from the shape and attachments of the mammalian tectorial membrane, which is thin and ribbon-like, and seems to have no contact at all with the tunnel rod cells (Tanaka and Smith 1978).

9.2.2 Hearing

Avian vocalization shows high complexity, and birds can compute precise temporal and spectral information in their vocalizations. Thus, the avian auditory system must exhibit the specializations necessary to allow them to process such complex sound stimuli (Kubke and Carr 2000). There are two cochlear nuclei where auditory fibers of the vestibulocochlear nerve terminate in birds: the nucleus magnocellularis and the nucleus angularis. A third nucleus, strongly associated with the cochlear nuclei is the nucleus laminaris where interaural time differences are first processed. Both nucleus angularis and nucleus laminaris project to the lemniscal nuclei and the inferior colliculus (Jhaveri and Morest 1982).

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10

The Cardiovascular System

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10.1 Introduction

The cardiovascular system of the chicken is best approached from an evolutionary perspective. Aves are a derived group of vertebrates that share many cardiovascular characteristics with other amniotes. Due to the familiarity of the mammalian cardiovascular system most readers possess, differences between chicken and mammalian cardiovascular systems will be emphasized. The morphological and functional characteristics follow the adaptive suite associated with cellular respiration in terrestrial vertebrates. The cardiovascular system is an assortment of connected tubes that propel and transport fluid to the sites of gas and nutrient exchange in the animal body. An almost linear development of complexity can be followed from non-tetrapod ancestral relations. Tetrapods display a double circulation system that comprises a heart (the pump) and vessels of diverse sizes (arteries, arterioles, capillaries, veins, and venules). The architecture and distribution of these structures will be the focus of this chapter. As with most characteristics of the chicken, their divergence from those of other tetrapods can be related to the adaptive suite of highly nutrient expensive flight.

Blood cells: please see Chapter 11, The lymphatic system.

10.2 Heart

10.2.1 Heart Topography

The chicken's heart is in the cranial part of the thoraco-abdominal cavity, to the right of the midline and between the second and sixth ribs. More specifically, the cardio-abdominal cavity is defined by pleuro-peritoneal and septal boundaries. The pericardial sac is a fibro-serous sac surrounding the heart (Figure 10.1) and in contact with the primary bronchi, syrinx, air sacs, and esophagus dorsally (pulmonary surface), the liver caudoventrally (hepatic surface), proventriculus caudo-dorsally, and the sternum-associated musculature and crop cranio-ventrally (sternal surface) (Nickel et al. 1977, p. 87). Phlebotomy (blood collection) may be achieved through multiple sampling locations (Greenacre and Morishita 2021, p. 506). Although not the preferred site when considering wellbeing of individuals, cardiac puncture has been done on anesthetized birds without showing deleterious effects (Cook et al. 1982) and is deemed a desirable source of blood during necropsy when compared to the thin walled (prone to hematoma) and mobile nature of the brachial vein (Schwartz and Bickford 1986).

These researchers detail a ventral approach, via the thoracic inlet using the keel as an alignment landmark, and a lateral approach, where the cranial extent of the sternum, anastomosis of the superficial pectoral vein, and the dorsal border of the pectoral muscles are used as landmarks.

10.2.2 Pericardial Sac

The pericardial sac and cavity are arranged like in mammals, which comprises a superficial and tough fibrous pericardium that is associated with a double layering of lubricating serosa. The parietal serous pericardium is found in close association with the outer fibrous layer. This serosal layer is a mesothelium (simple squamous epithelial tissue) which is continuous with the visceral serous pericardium (epicardium), reflecting cranially to cover the myocardium. The fibrous pericardium maintains the position of the heart via blending with the proximal adventitia of the great vessels and anchors to the ventral body wall. The three layers of the pericardium comprise the

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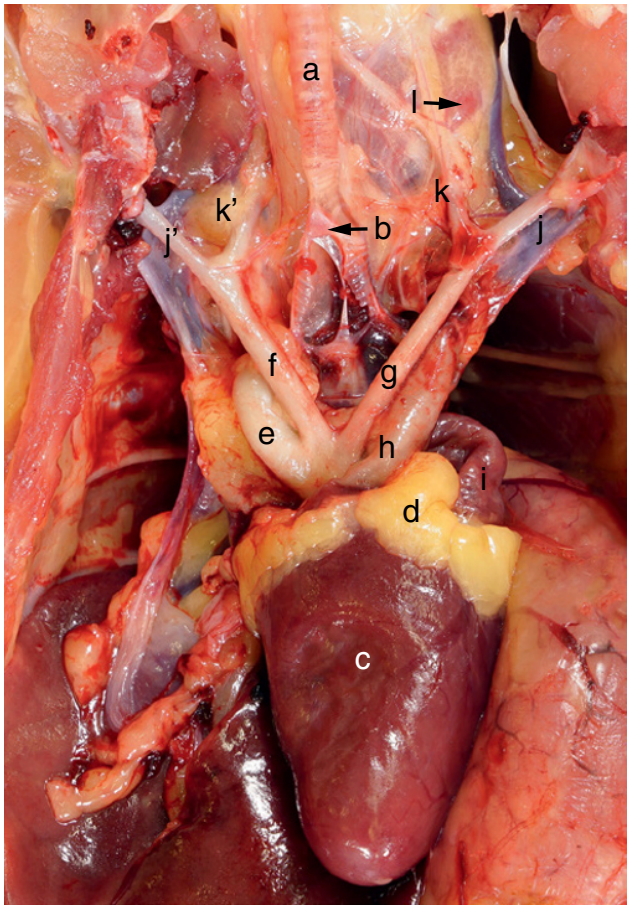

Figure 10.1 Chicken heart, ventral view, (a) trachea, (b) syrinx, (c) heart without pericardium, (d) fat on the coronary groove, (e) aorta, (f) right brachiocephalic trunk, (g) left brachiocephalic trunk, (h) pulmonary trunk, (i) left auricle, (j, j') left and right subclavian arteries, (k, k') left and right common carotid arteries, (l) thyroid gland.

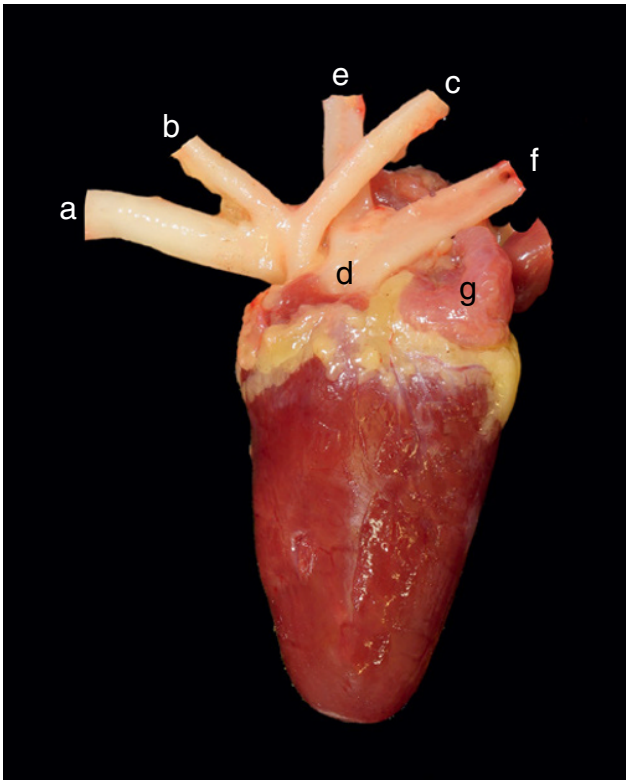

Figure 10.2 Chicken heart left lateral view, (a) aorta, (b) right brachiocephalic, (c) left brachiocephalic, (d) pulmonary trunk, (e) right pulmonary artery, and (f) left pulmonary a., (g) left auricle.

pericardial sac. The space between the two serosal layers is the pericardial cavity, which contains approximately 0.10 mL of pericardial fluid (Flick et al. 1963).

The great vessels emerging from and entering the pericardial sac, including the two brachiocephalic arteries (ventral most), dorsal aorta, pulmonary arteries, and veins, and three venae cavae (Figure 10.2), have varying degrees of coverage by adipose connective tissue depending on the body condition of the animal. The nerves and the lymphatic vessels of the heart course along with these vessels to reach or leave the heart.

10.2.3 Heart Surface Anatomy

The four-chambered chicken heart is an elongated cone-shaped organ (Figure 10.2), although dilated cardiomyopathy often changes the morphology and results in a more spherical shape in the faster-growing breeds (Olkowski et al. 2020). The right side of the heart is slightly concave, and the sternal surface is more rounded than the hepatic surface. The surface features of the chicken heart are like mammals. The myocardium appears shiny due to the translucent epicardium between subepicardial adipose connective tissue that resides in the coronary grooves. These shallow sulci are defined as coronary, dorsal interventricular, and ventral interventricular and carry cardiac vasculature. These vessels (coronary arteries) are often covered with adipose tissue. They originate from the aortic bulb. The blood flows into these vessels from stored energy in the elastic aorta when the valve closes the pathway from the left ventricle during ventricular systole. Early deep branches of the coronary arteries provide most of the blood supply to the atrial and ventricular walls. This differs from the mammalian flow, where the superficial arteries are primary, and explains why the sulci are shallow in the chicken. Thus, the left and right ventricles are best defined from one another by following the conus arteriosus from the pulmonary trunk (ventral interventricular sulcus). The dorsal interventricular sulcus resides on the hepatic surface and the coronary sulcus separates the atria from the ventricles. The apex of the heart is the best location to localize the left

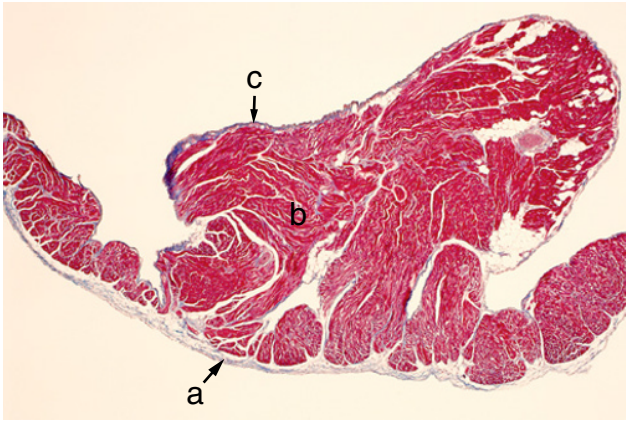

Figure 10.3 The left auricle wall sectioned at the pectinate muscle showing thin layer of epicardium (a), myocardium (b), and endocardium (c), trichrome stain.

ventricle and is associated with a fovea that corresponds to the extent of the interventricular septum. The superficial branches of the left and right coronary arteries primarily remain in the coronary groove. The left coronary artery supplies the conduction nodes via the interatrial artery. The cardiac veins are the vessels most apparent on the surface of the interventricular grooves.

The left and right atria of the heart are thin walled except in areas where bulbous muscular bands (pectinate muscles) exist, creating a fenestrated appearance when filled with blood (Figure 10.3). They are least visible in the lateral and caudo-dorsal views. The spaces between the pectinate muscles are the sinuses of the heart chambers. The ear-shaped appendages of the atria, the left and right auricles, are located dorsally.

10.2.4 Chambers of the Heart

Due to the persistent presence of the sinus venosus and the modified flow of blood from the pulmonary veins, six recognized spaces exist within the chicken heart. This should not be confused with terminology referring to the four chambers. The chicken possesses a four-chambered heart, otherwise quite like mammals in architecture.

10.2.5 Sinus Venosus

Despite being difficult to observe, due to damage inflicted when removing the pericardial sac, a sinus venosus is retained in the chicken. It receives blood from the right cranial and caudal vena cava. An inter-venous tubercle exists between these vessels, which functions to decrease turbulence as relatively deoxygenated blood enters and directs the blood into the atrioventricular valve. A sinoatrial valve prevents backflow into the thin-walled sinus venosus from the right atrium.

10.2.6 Right Atrium

The right atrium receives drainage directly from the left cranial vena cava, the dorsal cardiac, and the left cardiac veins. No coronary sinus exists in chicken. The valve separating the sinus venosus from the right atrium (sinoatrial valve) comprises the roof of this chamber. The crista terminalis is associated with the pectinate muscle of the cranial extent of this sinoatrial valve. In addition to the right auricle, a recess of the right atrium extends the chamber toward the aorta and pulmonary trunk. Separation from the left atrium is accomplished by the interatrial septum, which creates a funnel-shaped progression toward the semicircular right atrioventricular ostium due to its oblique orientation. The interventricular septum forms the medial floor of the atrium.

10.2.7 Right Ventricle

The right ventricle of the chicken heart is remarkably like that of the mammalian heart. It extends about two-thirds of the distance from the coronary sulcus toward the heart's apex. It surrounds a portion of the left ventricle and is shaped into a funnel ending in the pulmonary artery (conus arteriosus). It has a third to a quarter of the thickness of the walls in front of the left ventricle and has conduction fibers spanning from the interventricular septum to the parietal wall. Significant differences exist, from mammals, in that no chordae tendineae or papillary muscles are present with the modified right atrioventricular valve (see Section 10.2.10). The trabeculae carneae are lacking, and the interventricular septum is thin and conforms to the shape of the left ventricle.

10.2.8 Left Atrium

The left atrium receives left and right pulmonary veins, which in part join and invaginate to form a common pulmonary vein. This vein's right margin is fused to the ventral interatrial septum. The dorsal interatrial septum is thin and corresponds to the fetal perforated septum allowing shunting from the right atrium, like the fossa ovalis of mammals. A portion of this vessel extends into the right atrioventricular septum, providing blood directly into the left ventricle and defining a pulmonary chamber from the left atrium proper. The left margin of this invaginated vessel acts as a valve of the pulmonary vein to prevent backflow into it. Dorsally, the course of the left vena cava toward the right atrium provides support to the atrial myocardium.

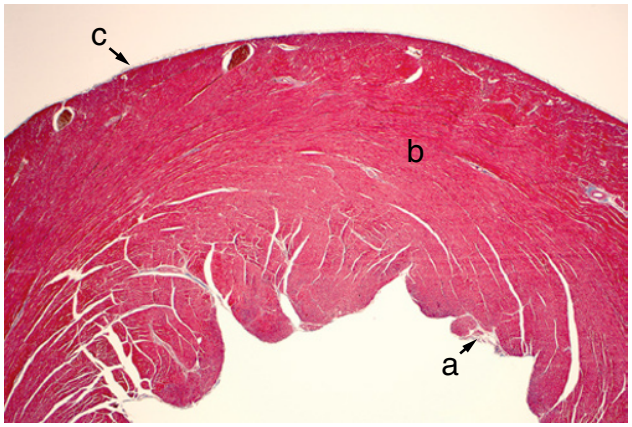

Figure 10.4 A cross section in the wall of the chicken heart at the left ventricular level showing (a) endocardium, (b) myocardium, and (c) a very thin epicardium (c), trichrome stain.

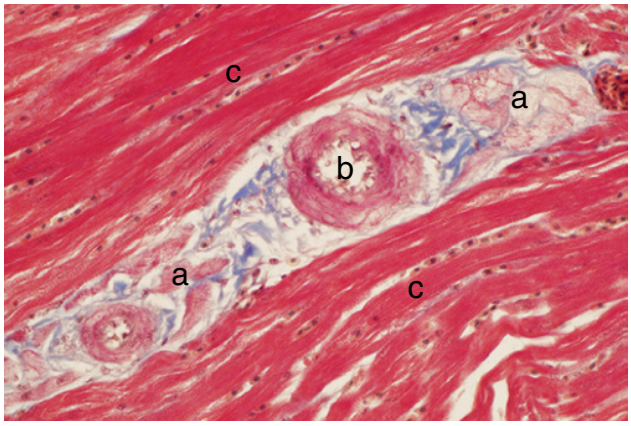

Figure 10.5 A section passing through the myocardium of the chicken shows (a) Purkinje fibers, (b) blood vessels, and (c), cardiomyocytes, trichrome stain.

10.2.9 Left Ventricle

The left ventricle of the chicken heart maintains the same organization and characteristics as the mammalian heart (Figure 10.4).

10.2.10 Atrioventricular Valves

The chicken's atrioventricular valves differ from those of mammals, associated with the maintenance of the increased heart rate (Lu et al. 1993a,b). The right atrioventricular valve is a singular structure of myocardial tissue, spiraling to account for the lack of the supraventricular crest. The left atrioventricular valve comprises three cusps and a cylinder of myocardium joins it to the aortic valve, allowing the outflow of the left ventricle to be constricted. An organized ring of Purkinje conduction fiber encircles these structures to account for the additional myocytes. The aortic, pulmonary, and two atrioventricular valves as well as the myocardium supporting cardiac skeleton, each consisting of fibrous connective tissue, are more robust than mammals.

10.2.11 Cardiac Skeleton

The chicken's cardiac skeleton is large and prevents inward collapse of the walls due to the force of high blood pressure during systole. It comprises fibrous connective tissue residing at the base of the ventricles and surrounding the atrioventricular ostia (openings) and the roots of the pulmonary trunk and aorta. Fibrous trigones (left and right) and rings (aortic, pulmonary, and right and left atrioventricular) provide surface area for the attachments of the atrial and ventricular myocardium and create a structure for the separation of atria and ventricles via the atrioventricular septum.

10.2.12 Conducting System

The chicken conducting system is composed of a sinus node (sinoatrial), atrioventricular node (AV), the bundle of His (left, right bundle branches), and an AV Purkinje ring as well as a special middle bundle branch which is unique to the avian heart (Lu et al. 1993a,b). The sinus node lies near the base of the lower portion of the right sinoatrial valve. Other researchers described it as between the orifices of the right cranial vena cava and the caudal vena cava (Kim and Yasuda 1979). The AV node is just above the tricuspid valve. The bundle of His is longer than in mammals (Lu et al. 1993a,b). The bundle of His descends into the interventricular septum to divide into right, left, and middle branches. Purkinje cells in mammals are modified cardiac muscle cells (cardiomyocytes) lightly stained and larger in size than cardiac muscle cells. In chickens, Purkinje-like cells are smaller and darker than those in mammals (Figure 10.5). They are present in the bundle of His and under the endocardium (Lu et al. 1993a,b). Other cells are also described in chicken heart like P and transitional cells.

10.2.13 Heart Arterial Supply

The chicken heart has two main arteries like the mammals. The right coronary artery arises at the sinus of the right semilunar valvula of the aorta. It divides into superficial and deep branches. It supplies the wall of the right atrium, a sizable portion of the septum, and the wall of the left ventricle.

The left coronary artery arises from the sinus of the left semilunar valvula of the aorta. The artery runs between the pulmonary trunk and the left atrium. It divides into two branches (superficial and deep). It enters the coronary

groove to supply the wall of the left part of the right atrium, part of the septum, and part of the left and right ventricles. It has a deep branch to supply the interventricular septum (Nickel et al. 1977, p. 90).

10.2.14 Venous Drainage of the Heart

There are four chicken cardiac veins, the great cardiac vein, middle cardiac vein, left circumflex vein, and cardiac venous plexus (Whittow 2000, p. 146). The great cardiac vein of a chicken is smaller than the middle cardiac vein. It has two segments, interventricular and basal. The cardiac orifice of the great cardiac vein is found either close to the left precaval ostium, or on the floor of the right ostium, or the right atrium. The great cardiac vein may pursue its usual course but finally opens into the right atrium in common with the termination of the left circumflex vein (Lindsay 1967).

The middle cardiac vein is the principal venous drainage from the left ventricular wall. It is located within the subsinuosal groove to open above the coronary groove. Many of the tributaries from the left aspect of the apex of the heart course horizontally beneath the epicardium to join the left apical radicle. The right apical radicle and the main trunk receive vessels from the adjacent part of the interventricular septum and the wall of the right ventricle (Lindsay 1967). Small cardiac veins open directly into the wall of the heart.

10.3 General Arterial System

10.3.1 Pulmonary Trunk

The pulmonary trunk carries low oxygenated blood that arises from the right ventricle. Once it leaves the left ventricle, it divides into the right and left pulmonary arteries (Figure 10.2). Each pulmonary artery enters the respective lung parenchyma to divide independently of the bronchial tree into smaller arteries until the terminal capillary beds within the parabronchi (König et al. 2016, p. 162). The pulmonary trunk is guarded at the junction with the right ventricle by three semilunar cusps (valvulae) to prevent regurgitation of the blood after systole. The base of the pulmonary trunk wall is dilated with a thin wall at the site of attachment of each cusp (valvula) (Getty 1975, pp. 1981–1982). The wall of the pulmonary trunk is thinner than the aorta because of the difference in blood pressure between the two vessels. However, both the aorta and the pulmonary arteries are elastic in classification, allowing potential energy to be stored to help propel blood after cardiac contraction. The cranial ramus of the pulmonary artery supplies the cranial one-third of the lung, along with the larger caudomedial ramus which supply the thick portion of the lung close to the vertebrae. The smaller caudolateral ramus supplies the rest of the lung parenchyma.

It is suggested that the distribution of pulmonary blood flow is mediated by local mechanisms arising in the airways (Weidner et al. 2012) or involves changes in the shape of vascular resistance within the inter-parabronchial arterioles (King and McLelland 1984, p. 3).

10.3.2 Systemic Arteries

Both ascending and descending aortae are elastic arteries. Most of the tissue type within the tunica media is elastic fibers. The internal elastic lamina of the aorta is difficult to discern because of the intensity of the close by elastic fibers associated with the tunica intima. Smooth muscle cells are interspersed between the elastic and collagen fibers within the tunica media (Figure 10.6). As the aorta descends caudally into the coelomic cavity it gradually changes into muscular at the division of the iliac arteries. The only exception is at the junction of the descending aorta and the origin of the renal arteries where an abrupt change from an elastic aorta to a muscular artery enters the kidneys. Most of the arteries within the chicken are like those in mammals of muscular artery classification, simply varying in size, and being further classified by their lumen diameter into large, medium, and small arteries (Figures 10.7–10.10). The small arteries terminate into arterioles that break into arterial capillaries to supply the tissue with nutrients and oxygen (Figure 10.11).

10.3.2.1 Cervical Region

Inside the thoracic region anterior to the heart, the aorta gives rise to both left and right brachiocephalic trunks. Each brachiocephalic artery originates from the ascending aorta and gives rise to a respective common carotid artery before terminating as the subclavian artery of that side.

10.3.2.2 Head

A considerable number of branches and anastomoses between the blood vessels in the chicken head result in the possibility of two-directional flow of the blood depending on the differential pressure within the opposing vessels.

Several cervical and cephalic locations have arteries surrounded by venous plexuses which can act as countercurrent heat exchange regions (Getty 1975, p. 1983).

The common carotid artery is a branch of the brachiocephalic artery and is classified as muscular, with a thick tunica adventitia (Figures 10.1 and 10.8). The internal carotid artery is a continuation of the common carotid artery. The continuation of the internal carotid artery is the

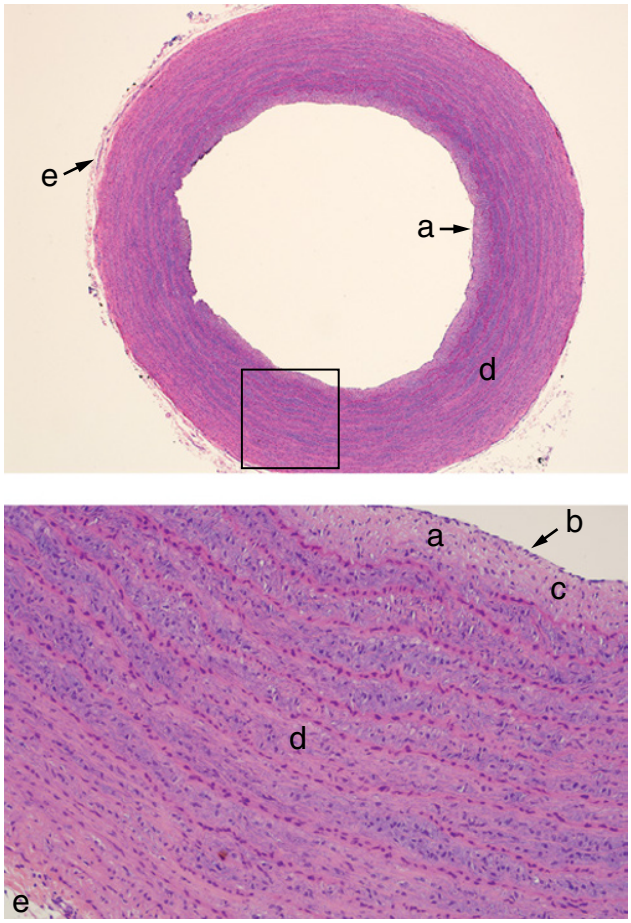

Figure 10.6 A cross section of the ascending aorta of chicken (two magnifications). Notice the three tunics in the wall (a) tunica intima with (b) endothelial cells (simple squamous), (c) subendothelial connective tissue, (d) thick middle layer, tunica media composed of elastic fibers and smooth muscle cells (e), extremely thin tunica adventitia, H&E.

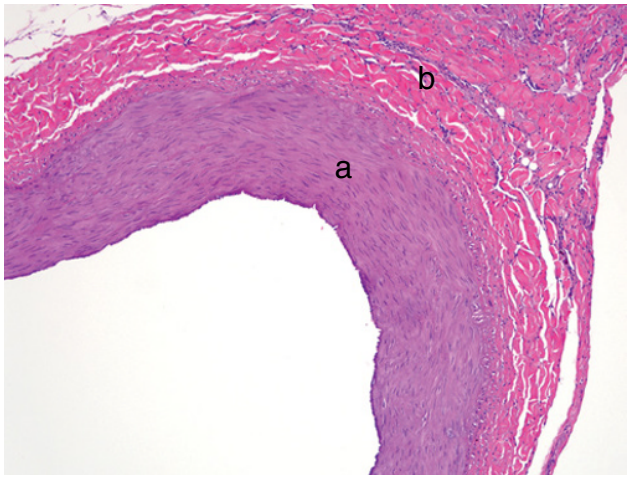

Figure 10.7 Large muscular artery with (a) thick tunica media as well as (b) thick tunica adventitia, H&E.

Figure 10.8 Common carotid artery of the chicken with (a) tunica intima, (b) tunica media, compared to (c) thick tunica adventitia, H&E.

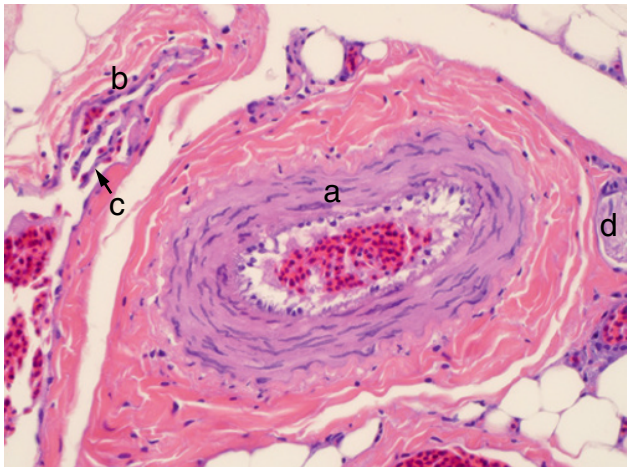

Figure 10.9 Medium-size muscular artery (a) and a collapsed vein (b) with a valve (c) and nerve fiber (d). H&E.

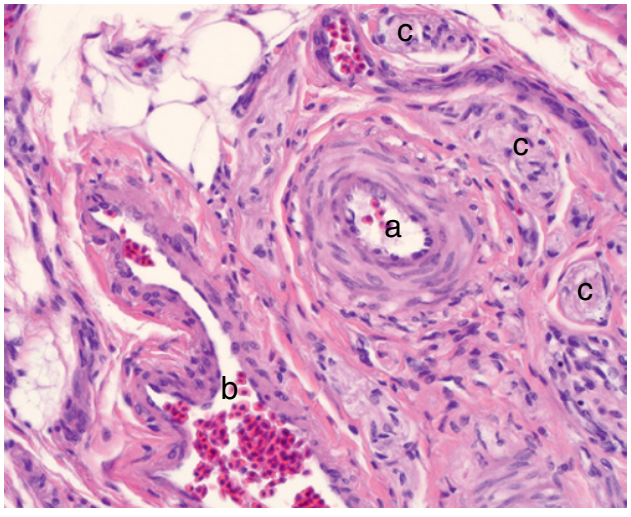

Figure 10.10 A small-size muscular artery (a) and a vein with red blood cells within its lumen (b) scattered nerve fibers (c), H&E.

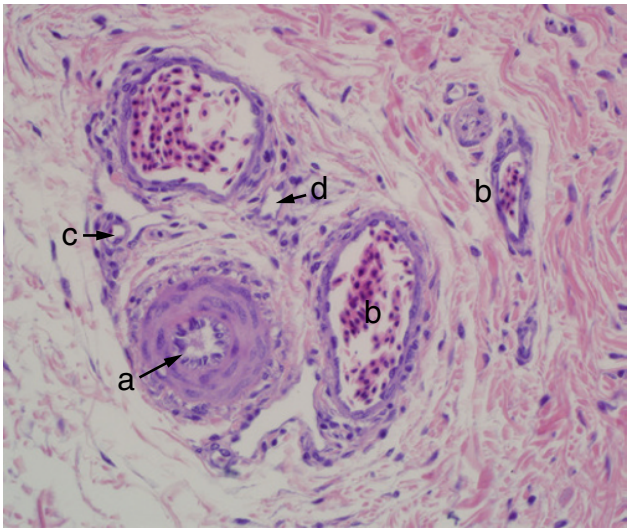

Figure 10.11 Terminal blood vessels showing (a) arteriole, (b) venule, within connective tissue mostly collagen fibers, (c) blood arterial capillary, and (d) venous capillary, H&E stain.

cerebral carotid artery. This artery supplies the brain and the meninges with one exception, the ethmoid artery which detaches from it and supplies the nasal cavity (Getty 1975, p. 1983). The internal carotid artery enters the cranium through the carotid foramen and contributes to the blood supply of the brain. The left and right cerebral carotid arteries branch to form a structure like that of mammals, including the cerebral arterial circle (circle of Willis).

The external carotid and the external ophthalmic arteries are the major blood supply to the head outside the cranium (Getty 1975, p. 1983). The external ophthalmic artery arises from the internal carotid artery medial to the external acoustic meatus (Getty 1975, p. 1985). In the caudolateral orbit, they form the ophthalmic rete mirabile which is surrounded by a venous plexus, and plays a role in countercurrent heat exchange mechanism.

10.3.2.3 Breast and Thoracic Limb

The main blood vessel that reaches the wing is the subclavian artery. It arises from the brachiocephalic trunk. It supplies the cranial and caudal pectoral muscles and lateral body wall. The subclavian artery changes name at the level of the scapulo-humeral joint into the brachial artery. The latter vessel branches into the radial and ulnar with superficial branches and a deep branch that forms the radial artery (Getty 1975, pp. 1988–1989). The basilic vein (cutaneous ulnar) is evident through the skin on the ventral side of the upper wing. The superficially located ulnar artery can be detected through the skin of the lower wing and above the fascia of the superficial digital flexor (Figure 10.12).

10.3.2.4 Spinal Cord

The cervical and the cranial thoracic portions of the spinal cord are supplied with blood from the ascending and descending vertebral arteries. The rest of the spinal cord, caudal to the base of the heart is supplied with blood by intersegmental arteries arising from the descending aorta. These intersegmental arteries result in the formation of the ventral spinal artery and paired dorsolateral arteries. They form an anastomosing arterial network that contributes blood to the entire spinal cord (Getty 1975, p. 1999).

10.3.2.5 Abdominal Viscera

10.3.2.5.1 Celiac Artery The celiac artery is the vessel that supplies the foregut. The organs included are the proventriculus, ventriculus (gizzard), upper part of the small intestine, liver, spleen, and pancreas (Getty 1975, p. 1990).

10.3.2.5.2 Anterior (Cranial) Mesenteric Artery The anterior mesenteric artery arises from the descending aorta to supply the midgut. It supplies the intestine at the duodeno-jejunal junction to the ileo-ceco-colic junction (Figure 10.13). Branches of both sides of the vessels anastomose with branches from the celiac and the caudal mesenteric arteries (Getty 1975, p. 1991).

The anterior mesenteric artery, which runs in the mesentery, supplies most of the intestinal tract. Its attachment is much more pendulous than the celiac or other visceral arteries and is much more affected by peristaltic movement of the viscera than other abdominal vessels (Ball et al. 1963) (Figure 10.13). The presence of an outer longitudinal muscle layer in part of the adventitia is reported to help in accounting for this increased degree of movement. The possible functional significance of this feature is discussed by other researchers on white-leg horn chicken. It is suggested that the function of the longitudinal muscle layer is to modulate the reactivity of the anterior mesenteric artery to vasomotor stimuli, and that may be particularly implicated in the cardiovascular response to sudden stress. The close association of this longitudinal muscle coat within the wall of the anterior mesenteric artery suggests that it may serve some function in controlling visceral blood flow (Bell 1969).

10.3.2.5.3 Posterior (Caudal) Mesenteric Artery The caudal mesenteric artery originates from the ventral surface of the descending aorta, caudal to the caudal lobe of the kidney, and is soon divided into cranial and caudal branches. These branches supply the rectum, cloaca, and cloacal bursa. Just as there are anastomoses among the branches of the cranial mesenteric artery, they exist among branches of the caudal mesenteric and celiac arteries (Campos et al. 2006). The branches of the posterior mesenteric artery supply the

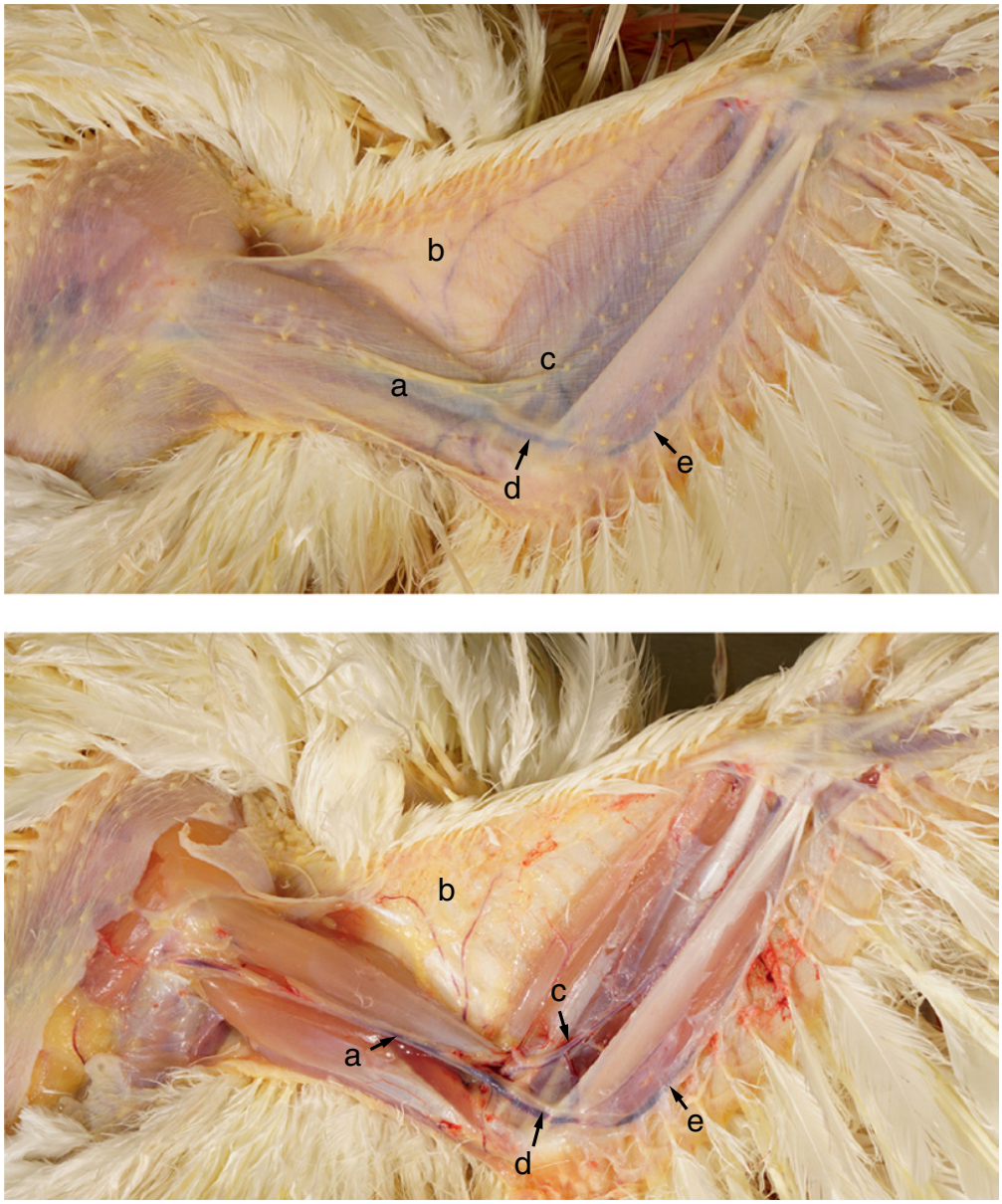

Figure 10.12 The ventral aspect of the chicken wing shows the course of the blood vessels and the site of blood collection, (a) brachial, (b) patagial vessels, (c) radial, (d) site of blood collection, and (e) ulnar.

caudal portion of the colon and cloaca which ramify in the cranial portion of the cloaca and anastomose with the cloacal branches of the internal pudendal artery (Khalifa and Ali 2014).

10.3.2.5.4 Blood Supply of Kidneys Renal arteries exist in three pairs in the chicken because of the size and segmentation of the kidney. The anterior renal arteries arise from the abdominal aorta opposite to each other with few exceptions (Siller and Hindle 1969). The anterior renal artery has a cranial branch which supplies the testis in male and the ovary in female, and caudal branches which supply the anterior lobe of the kidney. The caudal branch of the anterior renal artery anastomoses with the middle renal artery. The middle and the caudal arteries arise from the sciatic to supply their respective lobes. Variations may exist in these two arteries. The renal arteries undergo several divisions, giving the intralobular arteries which, in turn, give the afferent arterioles to the glomeruli. Each lobule has several intralobular arteries. Afferent arterioles

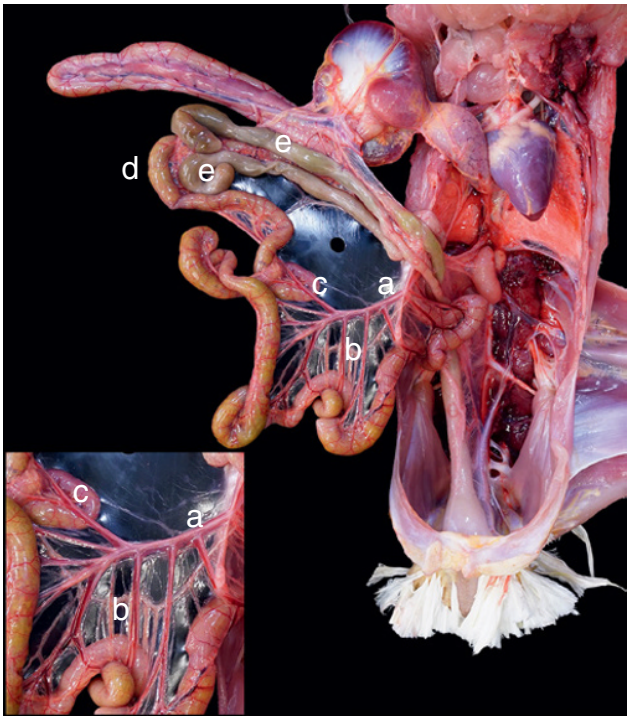

Figure 10.13 Branches of cranial mesenteric artery in chicken. (a) Cranial mesenteric a., (b) jejunal branches, (c) ileal branch, (d) ileum, and (e) cecum.

branch into glomerular capillaries followed by efferent arterioles which leave the glomeruli.

The glomerular capillary architecture of both nephrons, those that include or those that a lack Henle's loop, was examined by electron microscopy in *Gallus*. It was reported that there is no significant difference in the diameter of the glomerular capillaries between the two types of nephrons; however, the diameter of the afferent arteriole is significantly larger than that of the efferent arteriole. Based on size alone, the predicted blood flow rate in the efferent arteriole is 20% that of the afferent arteriole in *G. gallus*. There is no significant difference in the volume density of the glomerular capillaries between the two types of nephrons. Overall, the avian glomerular capillaries of the chicken are less complex than those of mammals (Casotti and Braun 1995).

Outer cortical glomeruli are small and therefore the blood capillary arrangement is simple (few loops). The larger glomeruli (juxtamedullary) have more complex branching of capillaries from the afferent arterioles before the formation of the efferent arterioles. This branching are not comparable to the mammalian tufts of capillaries within the glomeruli. Efferent arterioles only give off long capillaries which form a complex network of cortical peritubular sinuses. In the juxtaglomerular region, the efferent arterioles supply the vasa rectae of the medullary tracts.

Vasa rectae arterioles arise from the efferent arteriole and descend into the medulla to form the peritubular capillaries. The peritubular capillaries join to result in the formation of venules. These venules ascend into the corticomedullary region to drain into the interlobular veins (King and McLelland 1975, p. 177). The arteriole has one or two smooth muscle layers in its tunica media while the venule has no smooth muscle cells with a rare exception where only one smooth muscle cell layer is present. The arteriole and the venule are satellite vessels; therefore, one can differentiate the two by their cross-sectional diameters. The smaller and more circular one is the arteriole, due to the thickness of the tunica media, and the one with the irregular lumen and larger diameter is the venule. However, King and McLelland (1979, p. 217) stated that histologically, arterioles and venules are difficult to discern from each other under a light microscope.

10.3.2.5.5 Blood Supply to Gonads In the chicken the descending aorta within the abdominal portion of the coelomic cavity gives rise to two large arteries (left and right ischiatic). These vessels branch into the right and left middle and posterior renal arteries. The cranial renal artery gives off the testicular artery (Getty 1975, p. 1993), though rarely, an accessory testicular artery originates directly from the descending aorta. In the female, the left ovary and oviduct persist after the involution of the right ovary and oviduct; therefore, the left cranial renal artery gives rise to the left ovarian and oviductal arteries toward the left ovary and oviduct. The middle oviductal artery arises from the ischiatic artery while the caudal arises from the left pudendal artery (Getty 1975, p. 1993). In addition, the hypogastric artery originates close to the origin of the renal arteries and supplies the utero-isthmus region of the oviduct (Freedman and Sturkie 1963).

10.3.2.6 Aorta Termination

The descending aorta beyond the point of caudal mesenteric artery origin is termed median sacral artery (Freedman and Sturkie 1963). The aorta then branches into two left and right internal iliac arteries. Each of these vessels gives rise to pelvic and pudendal arteries on both sides.

10.3.2.6.1 Pelvic Limb The two main vessels which supplies the pelvic limb are the external iliac and the ischiatic arteries. Three vessels branching from the above mentioned arteries contribute to the pelvic limb blood

Figure 10.14 Chicken hindlimb showing the metatarsal blood vessel which is usually used for blood collection.

supply, the circumflex femoral, femoral, and pulvini (pubic) arteries. The pulvini artery runs along the pubic bone (Koch and Rossa 1973, p. 112). The ischiatic supplies the caudal and medial femoral muscles and the popliteal space. Branches of ischiatic anastomose with the femoral off the external iliac to form the popliteal artery. From this anastomosis, the anterior and medial tibial arteries originate. Medial tibial supplies the gastrocnemius muscle, the skin of the tibial region, and its feather follicles. It extends distally to supply the leg and the toe (Koch and Rossa 1973, p. 112).

The cranial tibial artery is the largest terminal branch of the popliteal artery. It continues passing through the interosseous space at the upper one-third of the tibia and continued as the common dorsal metatarsal artery (Figure 10.14) (Kang et al. 2017).

10.4 Venous System

10.4.1 Pulmonary Veins

Highly oxygenated blood is collected from the capillary beds of the lungs by the left and right pulmonary venules, which are tributaries to the pulmonary veins. These veins open into the left atrium in separate ostia (openings). (Nickel et al. 1977, p. 99).

10.4.2 Systemic Veins

10.4.2.1 Cranial Vena Cavae

The chicken has one caudal and two cranial vena cavae. These are large, thin-walled, passively functioning, and easily collapsible veins. The cranial vena cavae collect blood from the head, neck, wing, and chest wall. Left and right cranial vena cavae have symmetrical tributaries. The confluence of the vertebral, subclavian, and external jugular veins results in the formation of the cranial vena cava. Both left and right external jugular veins are superficially located under the skin.

10.4.2.2 Caudal Vena Cava

The caudal vena cava collects blood from the hindlimbs, the pygostyle, the kidneys, and the abdominal organs. The tributaries to the caudal vena cava are as follows: hepatic (left, right, and small middle branches), testicular or ovarian, and external iliac (King and McLelland 1975, p. 102). Histology of the vein shows an endothelial layer like any other vessel with a subendothelial layer (intima), few smooth muscle cells in the middle layer, and connective tissue in the outermost layer (adventitia) with elastic fibers. The elastic fibers maintain their tension and elasticity. The media is mainly represented by smooth myocytes located at the periphery of the vein in the form of complex intertwisted oblique-circular and spiral fibers with little connective tissue among them (Fomenko, and Pervenetskaya 2020).

10.5 Sites for Blood Collection

The brachial wing vein (basilic or cutaneous ulnar vein) is the most common site for blood collection in an adult chicken (Figure 10.12). It can be accessed near the elbow. It runs between the biceps and triceps muscles and bifurcates just proximal to the elbow. Blood should be collected at the bifurcation. Two jugular veins are present, but the right side of the chicken's neck allows for faster collection of larger volumes of blood (Figure 10.15). The vein is very mobile and there is a large amount of subcutaneous space, making the site prone to hematoma formation. The medial metatarsal vein in the lower leg can be used to collect blood. It is surrounded by muscles, minimizing the risk of hematoma formation, but can be difficult to locate, especially in an adult bird (Kelly and Alworth 2013).

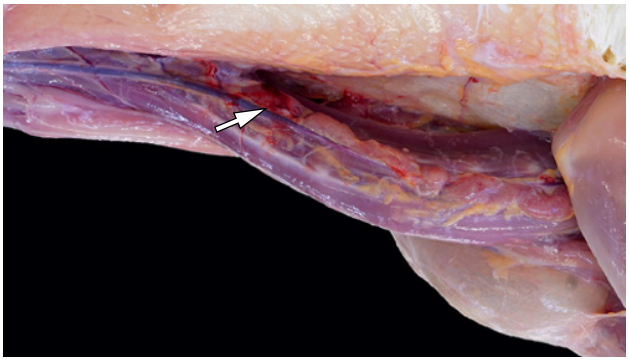

Figure 10.15 Cervical region of the chicken showing the left external jugular vein arrow.

10.6 Hepatic Portal System

There are two hepatic portal veins in birds. The left portal vein drains the stomach, and the right drains the remainder of the stomach, spleen, small, and large intestines. The large intestine is drained through the caudal mesenteric vein (coccygeo-mesenteric). This vein drains into the hepatic portal and renal portal systems. A difference from the mammalian hepatic portal vein is the two tributaries, one from each lobe of the chicken liver (King and McLelland 1975, p. 102).

10.7 Renal Portal System

In addition to the arterial system supplying the glomerular capillary loops, there is a unique renal portal system that carries afferent venous blood from the capillary network of the legs to the interlobular veins (Pervenetskaya and Fomenko 2018). It passes through the inter-tubular sinuses where it is mixed with post-glomerular arterial blood to be drained by the intralobular efferent veins (Akester 1967). Details of the renal portal sphincter regulation and control can be found in Johnson (1979), who reviewed the existing literature (Figures 10.16 and 10.17).

10.8 Carotid Body/Carotid Sinus

In contrast to the mammalian carotid bodies, in the chicken they are small rounded to oval, and located at the entrance of the thoracic cavity along to the parathyroid gland and ultimobranchial body. However, the location may vary in relation to the surrounding structures (Hodges 1974, p. 230). The carotid body, thyroid, parathyroid, and ultimobranchial glands are situated along the common carotid artery. Glomus cells are widely distributed not only in the carotid body but also in the wall of the common carotid artery and around the common trunk and its branches (Kameda 2002). The glomus cells of the chicken carotid body exhibit intense immunoreactivity for serotonin, tyrosine hydroxylase, and chromogranin A. (Kameda 2002). Due to its location, the carotid body is innervated by branches from the vagus and the recurrent laryngeal nerves (see Chapter 8, Endocrine System).

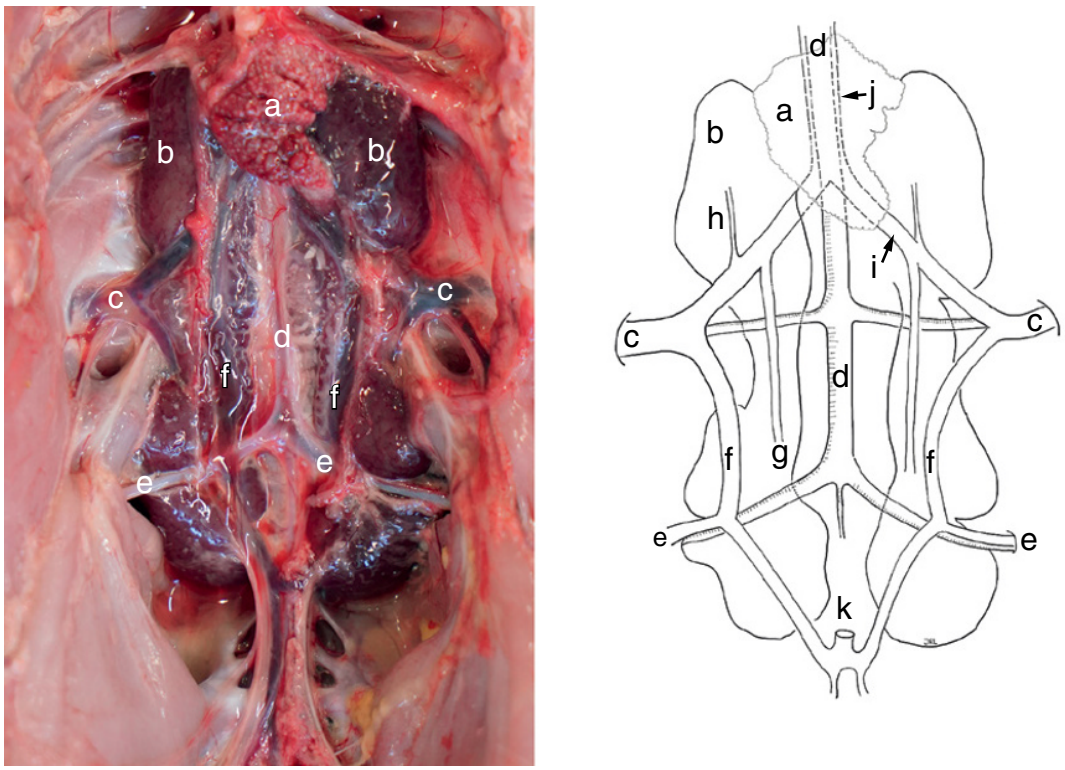

Figure 10.16 Ventral view of the abdominal coelomic cavity showing the blood vessels close to the gonads and the kidneys. (a) Developing ovary, (b) right and left cranial renal lobes, (c) external iliac v., (d) aorta, (e) ischiatic a. and v., (f) caudal renal portal vein, (g) caudal renal v., (h) cranial renal v., (i) common iliac v., (j) caudal vena cava (more ventral and larger than aorta), and (k) caudal mesenteric v.

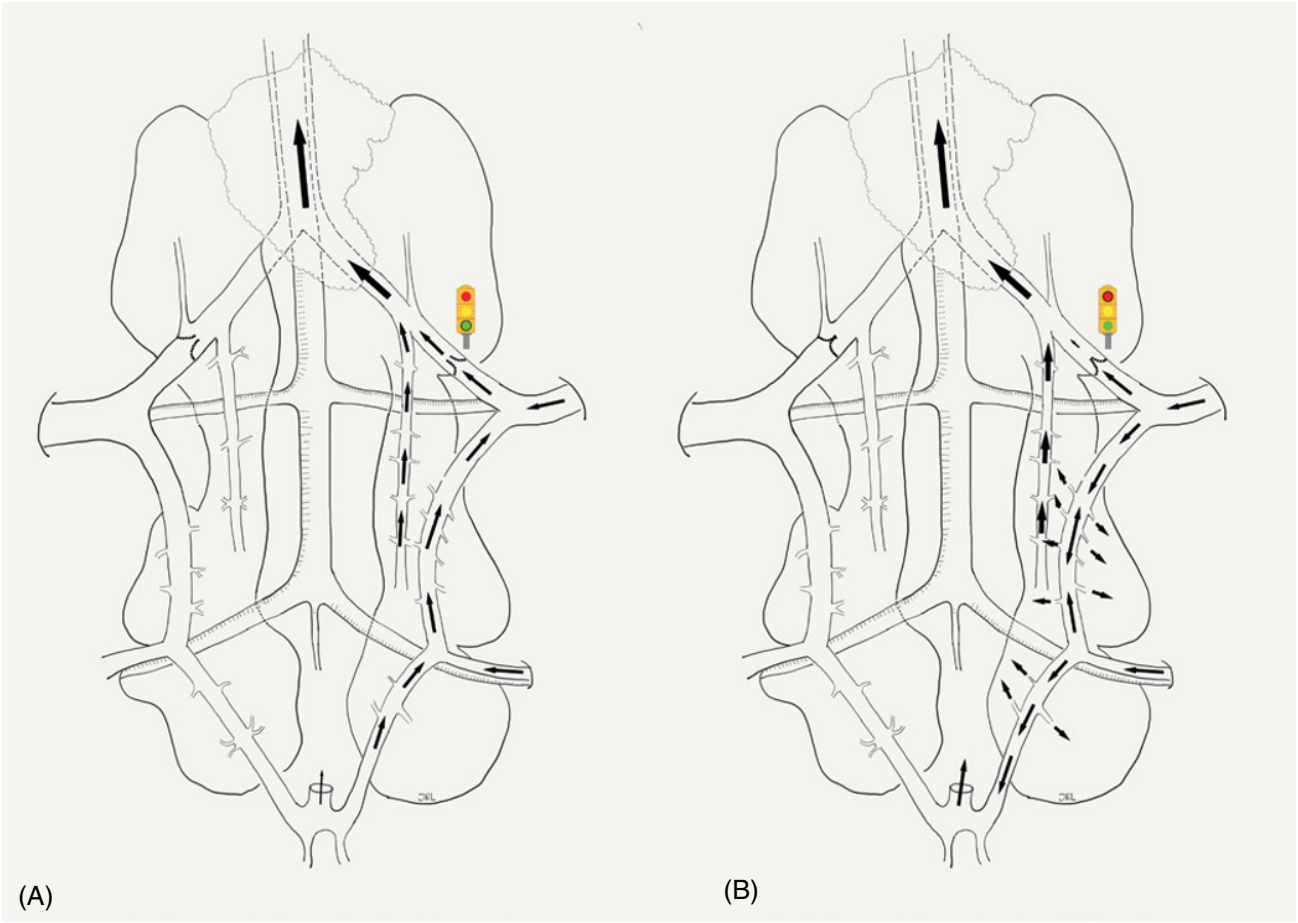

Figure 10.17 The illustration portrays the blood flow within the chicken's renal portal system, influenced by two distinct autonomic nervous system stimuli. The thickness of the arrows indicates an increase or decrease in blood flow. Under sympathetic control (A), the valve (indicated by dotted lines) remains open (green light), allowing blood from the caudal mesenteric, ischiatic, and external iliac veins to flow toward the caudal vena cava through the common iliac veins. In contrast, under parasympathetic control (B), the valve (dotted lines) is closed (red light), diverting the blood towards the kidneys and the caudal mesenteric vein, which then reaches the hepatic portal vein. The branches of the caudal portal renal veins enter the kidney, forming the peripheral interlobular veins that lead to the capillary network surrounding the nephrons. Peritubular vessels facilitate renal tubular secretion and reabsorption, then ultimately converge into the caudal renal veins, which join the common iliac veins.

The carotid sinus in mammals is located at the bifurcation of the common carotid artery, while in chicken the area homologous to the mammalian carotid sinus is not found but is located at the entrance of the thoracic cavity along the common carotid artery distal to the subclavian artery and proximal to the vertebral artery (Adams 1958).

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11

The Lymphatic System

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11.1 Introduction

The ability of the lymphatic system to recognize foreign molecules through its cells is a relatively complicated process. The lymphatic cells begin to appear at the early stage of embryonic development of the chicken (day 9 or 10 of incubation) (Owen 1972). They migrate from the yolk sac and the liver to populate the two primary lymphatic organs, the bursa of Fabricius and the thymus. Once inside these two primary lymphatic organs, they undergo a clonal expansion within the liver and the bursa of Fabricius. The secondary lymphatic organs include the spleen, lymph nodes (when present, absent in chicken), mucosal or gut-associated lymphoid tissues (MALT and GALT), and scattered cell aggregates without organization elsewhere in the animal body. The early lymphocytes either become immunocompetent B lymphocytes within the bursa of Fabricius (humeral mediated immunity) or populate the thymus to become immunocompetent T lymphocytes (cell-mediated immunity). After this stage of development, both cells leave the primary lymphatic organs to populate the secondary organs and will be easily detected inside the blood circulation.

It is not possible to differentiate between B and T lymphocytes under light microscopy using routine staining methods. They can be differentiated with immunohistochemical methods.

The functions of the lymphatic system are (i) carrying large molecules from the interstitial tissue back to the general circulation, acting as a transporter of lipids from the intestinal villi (lacteal), (ii) collecting waste substance from the interstitial spaces, and (iii) playing a role in controlling infection because of its immune function. However, the immune response should be considered the main function in most other body systems in addition to removing the waste substances.

The absence of lymph nodes in chicken influenced the distribution of dendritic and macrophages along with certain body systems. Many macrophages and dendritic cells are usually present in the mucosa of the avian large airways. The airflow within the chicken respiratory system influences the deposition of antigen along the course of the inspired air. This dictates the distribution of macrophages and dendritic cells accordingly (de Geus and Vervelde 2013). Terms used within this chapter followed the Handbook of Avian Anatomy as close as possible (Baumel et al. 1979).

11.2 Cells of the Lymphatic System

11.2.1 Lymphocytes

All lymphocytes are white blood cells (leukocytes) and are also called agranulocytes because they lack granules within their cytoplasm. They comprise 75% of the circulating leukocyte count in normal domestic fowl (Lucus and Jamroz 1961). Lymphocytes, in general, are rounded with a large nucleus occupying most of the cell. One can hardly see a small rim of cytoplasm surrounding the nucleus under the light microscope. Cell nuclei, in general, have either compact dark chromatin (heterochromatin) or less compact lightly stained chromatin (euchromatin). Lymphocyte in circulation and in organs when not activated has compact dark heterochromatin. This kind of chromatin indicates inactivity while the euchromatin is present within the active cells like the proliferating B cells inside the germinal center (Figure 11.1).

11.2.1.1 Small-Size Lymphocyte

A small-size lymphocyte is round and almost the size of a red blood cell in mammals (around 6 μm in diameter). The red blood cell in chicken is oval with an elongated nucleus;

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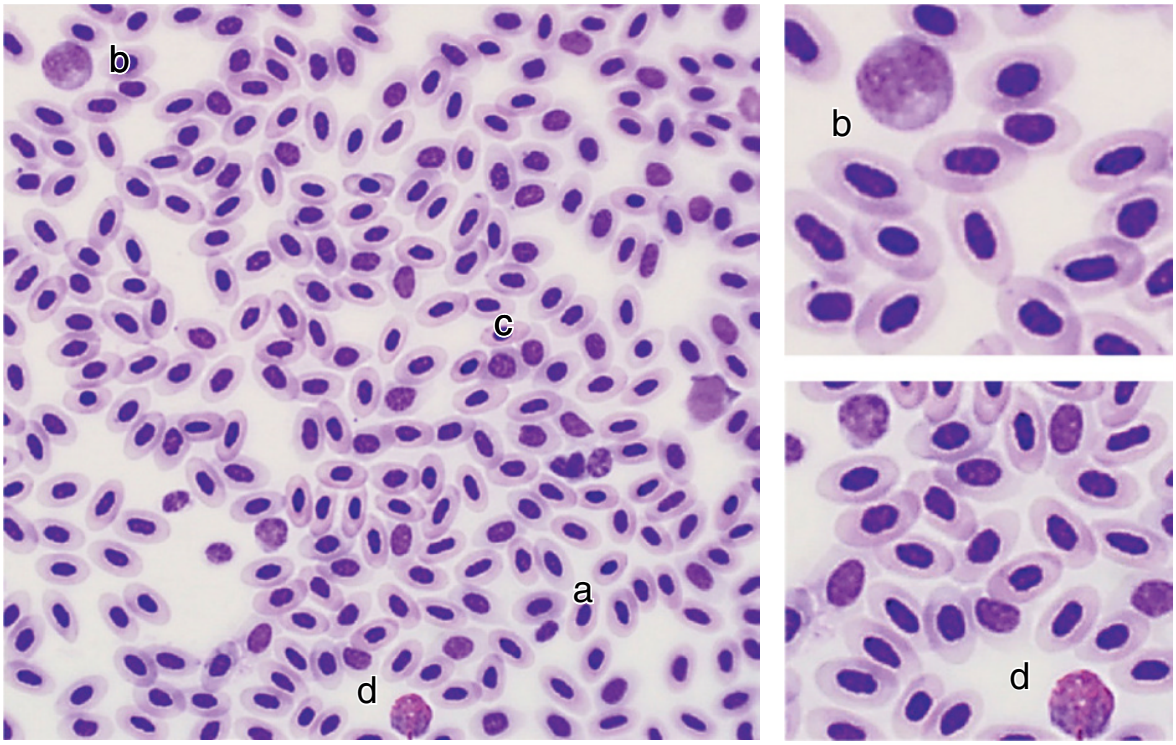

Figure 11.1 Chicken blood smear (stain) showing RBCs (a), monocytes (b), lymphocyte (c), and eosinophil (d).

therefore, it is difficult to make an easy comparison in size with the small lymphocyte. The small lymphocyte has a very thin rim of cytoplasm around the darkly stained nucleus because of the condensed chromatin. They are present in the circulatory system and dominate the thymus-dependent regions. No obvious immunoglobulin presents on their surfaces.

11.2.1.2 Medium-Size Lymphocyte

The medium-size lymphocyte is between small and large lymphocytes as the name indicates. This cell may have pseudopodia projections and less condensed chromatin compared to the small lymphocyte (King and McLelland 1979).

11.2.1.3 Large-Size Lymphocyte

A large-size lymphocyte is very close in size to the monocyte with the difference of always having a round nucleus and less cytoplasm when compared to the kidney-shaped nucleus of the monocyte. Eosinophilic cytoplasmic spheres granules are occasionally seen inside the large lymphocyte cytoplasm. These granules of unknown function are called azurophilic granules (Campbell 1988). A large lymphocyte is present within the germinal centers of the spleen white pulp and the bursa of Fabricius. It bears immunoglobulin on its surface and that is why the term bursa-dependent cell is used.

11.2.1.4 Naive Lymphocyte

A naive lymphocyte is a lymphocyte that has not encountered an antigen. This cell is found normally in the circulating blood, lymph vessels, and in tissues almost all over the animal body with few exceptions. The naive cell could be B or T lymphocyte.

11.2.1.5 B Lymphocyte

B lymphocyte is a mononuclear cell produced inside the bone marrow. It is the precursor of the plasma cell that produces immunoglobulin (Ig) in response to antigen exposure. It contributes to the humoral immune response through its indirect involvement in the production of antibodies (Abs). B cells respond to cell-free and membrane-bound antigens. B cells with low antigen immunoglobulin affinity are induced to apoptosis. B lymphocytes have an antigen receptor with a hydrophobic tail in the form of an antibody (Ab) anchored into the cell membrane. The process of differentiation of the B cell to plasma cell includes splicing of the mRNA which is responsible for coding of the hydrophobic tail. Thus, removing the coding part for the hydrophobic region, the antibodies produced by the plasma cells will not be anchored to the surface membrane and released into the surrounding tissue (Cross and Mercer 1993). Once exposed to an antigen (Ag), B lymphocyte transforms into a plasma cell, usually inside the spleen, and secretes

immunoglobulin (Ig). The immunoglobulins in chicken can be IgA, IgM, and IgY or chicken IgG (Leslie and Clem 1969, 1970). IgD and IgE are also reported to be present in chicken (Chen et al. 1982; Burns and Maxwell 1981). If contact with the antigen happens inside the lamina propria (loose connective tissue) of any tubular organ, then the immune response will probably be local to that region.

11.2.1.6 Plasma Cell

When a B lymphocyte is exposed to a foreign antigen, it will be stimulated and become a plasma cell. This cell is larger than the original B lymphocyte and has several identifying characteristic features. It has an eccentric nucleus, cart-wheel appearance arrangement of the chromatin, and presence of negative Golgi image. Because of the activity of plasma cells, the cytoplasm has many endoplasmic reticulum and Golgi apparatuses and that is why the presence of the negative Golgi image (lightly stained space) on the apical surface of the nucleus. Plasma cells have a major role in the immune response at local and systemic levels.

11.2.1.7 T Lymphocyte

Immunocompetent circulating T lymphocytes do not synthesize antibodies and once interacted with an antigen, it releases certain mediators. This response is called cell-mediated immunity. In general, any antigen that results in response of antibodies (production) requires the cooperation of the macrophages, B cell, and T cell. Also, T cells provide the population of recirculating lymphocytes which are found predominantly in the periarteriolar sheath of the spleen, the so-called thymus-dependent area (Hammer 1974).

11.2.1.7.1 T Helper Lymphocyte T helper lymphocyte is one of the most important cells in the adaptive immune response through the activation of B cell and induction of phagocytosis by the macrophage. Thus, one can state that the T helper is the initiator of the adaptive immune response. T cells respond to cell-bound antigens.

11.2.1.7.2 T Toxic Lymphocyte (T-Killer, Cytolytic) T toxic cells can destroy cells that are exposed or infected with a pathogen. Like any other white blood cell, T toxic cell has lytic enzymes within the granules with specific lysosomes inside its cytoplasm. Once it recognizes an antigen on the surface of a sick cell, it releases the granules.

11.2.2 Monocyte

The monocyte is a short-lived cell in the circulating blood. It originates in the bone marrow and becomes a macrophage or dendritic cell inside the tissue. It is a large agranulocyte cell (approximately 12 μm in diameter) present inside the blood circulatory system including the lymphatic. Monocyte has a kidney-shaped or rounded nucleus. It usually has foamy cytoplasm (presence of several vacuoles within the cytoplasm) which indicates the activity of the lysosomes. Monocyte is the source of macrophage and clasts like osteoclasts. Monocytes can engulf bacteria, particles, or damaged cells and digest them by the lysosomes within the cytoplasm (Maxwell and Trejo 1970; Maxwell 1974) (Figure 11.2).

11.2.3 Macrophage, Resident, and Wandering

The macrophage is a monocyte inside the tissue and histiocyte is another name used frequently to indicate macrophage inside the connective tissue. While the dendritic cell is used for cells present in the epidermis, lymphatic organs, and tonsils. The cell plays a big role in the immune response through its antigen-presenting capability. They may have several names depending on which organ they populate like the Kupffer cell in the liver, dust, wandering, and alveolar macrophage in the lung.

11.2.3.1 Dendritic

The dendritic cell has multiple cytoplasmic projections, and it is either blood or bone marrow in their origin. In both cases, this cell plays an integral role in the immune response and that is why it has a wide distribution in the animal body. The sites of distribution of the dendritic cells are inside the gastrointestinal tract and other body systems associated with the tonsils. The cells have a great ability to deliver antigens to T lymphocytes (Sutton et al. 2021). The cell can be detected with the routine stain as it appears with lightly stained cytoplasm and could be anywhere within the epithelium or the tonsils. Also, immunohistochemical staining will detect this cell. Resident dendritic cells originate from the mesenchyme while migrating dendritic cells originate within the bone marrow.

11.2.3.2 Microfold (M)

Microfold (M) cell is called M cell because of the number of folds on its apical surface seen under a transmission electron microscope. This cell is located between the simple columnar cells of the lining epithelium of the gut or elsewhere on top of the GALT or the MALT. Tight junctions connect M cells to the adjacent simple columnar epithelium within the cecal tonsils (Kato et al. 1992) and Meckel's diverticulum (Jeurissen et al. 1999).

11.2.4 Reticular Cell

The reticular cell is a fibrocyte-like present within most of the lymphatic organs including lymph nodes, spleen, and

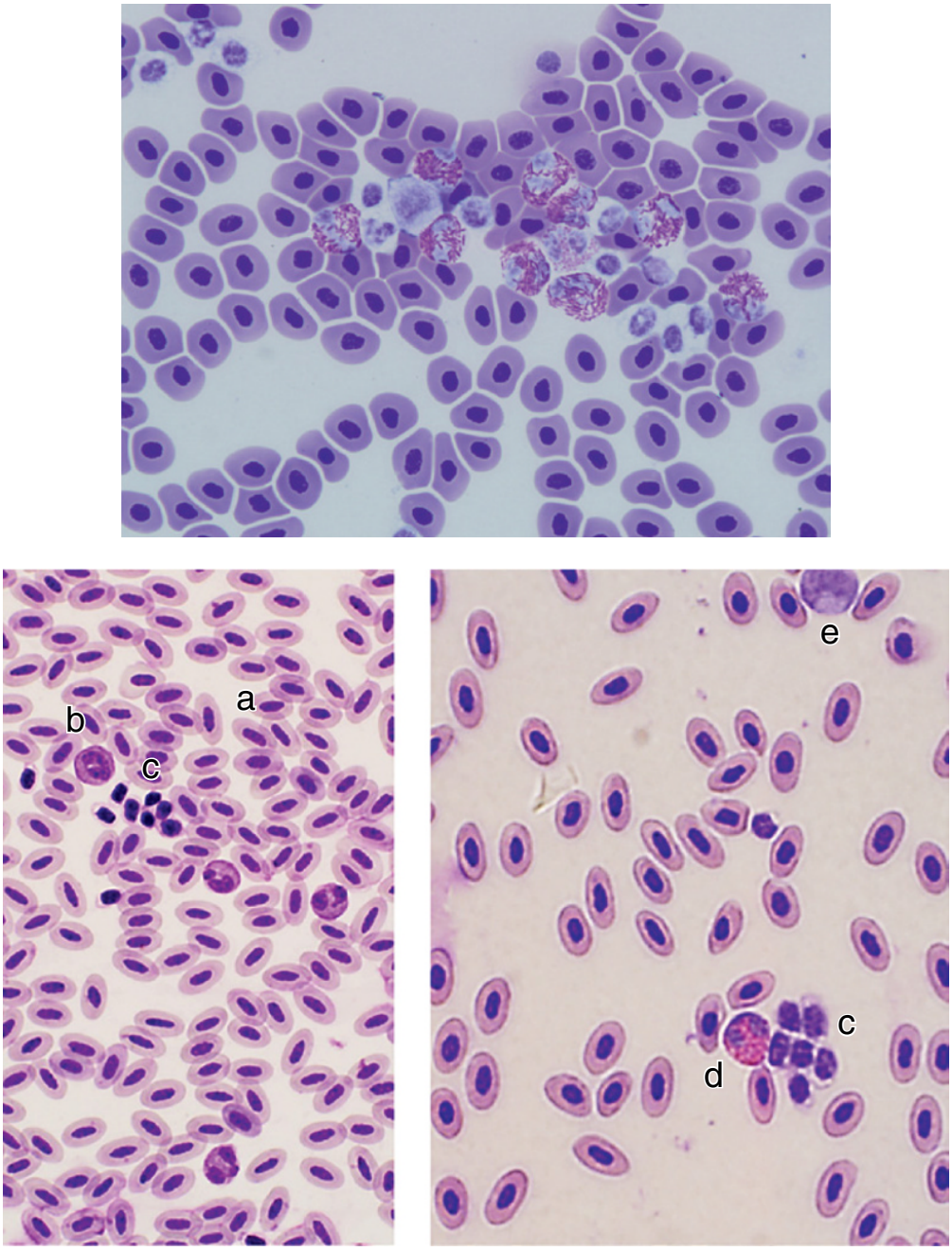

Figure 11.2 Chicken blood smear (stain) showing RBCs (a), heterophils (b), platelets (c), eosinophil (d), and monocyte (e).

lymph follicles. It produces reticular fibers through the production of special collagen fibers (type III). The reticular cell intricates fine fiber interconnect, forming a meshwork to support the lymphatic and other cells passing through. It is important to differentiate it from the reticulocyte, the immature red blood cell within the circulatory system.

11.2.5 Epithelial-Reticular

Epithelial-reticular or thymic epithelial is the structural cell within both the cortex and medulla of the thymus. It forms the structural meshwork with similar cells that promote lymphocyte proliferation and ensure no T lymphocyte escapes unchecked. It is a self-antigen presenter cell (Anderson and

Jenkinson 2001). It has long cytoplasmic processes which aid in helping to separate the growing lymphocytes from foreign antigen escaping from the blood circulation while presenting self-antigen on major histocompatibility molecules (Alexandropoulos and Danzl 2012).

11.3 Common Terms Used with the Lymphatic System

11.3.1 Capsule

The capsule of an organ or structure is usually dense irregular connective tissue with a few exceptions where one finds smooth muscle cells within the capsule-like in the spleen.

11.3.2 Trabecula(e)

Trabecula is an extension of the connective tissue capsule into the organ, separating the organ into lobes (gross anatomical term) or lobules (microscopic term). Blood vessels, nerves, and lymph pass through these trabeculae to reach the organ parenchyma.

11.3.3 Cortex

The cortex is an organized portion of an organ that is usually located in the outermost portion of it. It could be organized in the form of dense cells like in the thymus and bursa of Fabricius, or in the form of lymphatic follicles in case of lymph nodes or lymph nodules.

11.3.4 Medulla

The medulla is the inner part of an organ that is usually lightly stained and contains cords of lymphatic cells and blood vessels embedded within the epithelial-reticular meshwork in the thymus and within the reticular meshwork within the spleen and the lymph nodes.

11.3.5 Lymphatic Nodule (Follicle)

The lymphatic follicle or nodule is an organized aggregate of lymphocytes, mainly B cells, present in the lymph node cortex, spleen, and in scattered lymphatic structures, for example GALT.

11.3.6 Primary Lymphatic Follicle

A primary follicle is one that has not been yet exposed to an antigen and there is no activity of proliferation and cloning within it. It appears darkly stained because of the presence of many inactive B cells, reticular cells, and macrophages.

11.3.7 Secondary Lymphatic Follicle

The secondary lymphatic follicle is the one that has been exposed to an antigen; therefore, B cells are proliferating in large numbers within the center of the follicle. Thus, the follicle looks lighter in color because the spaces between the dividing cells and the active cell nucleus are euchromatic which looks lighter under routine staining of the tissue.

11.3.7.1 Germinal Center

The germinal center is a lightly stained region within the center of the lymph follicle present inside the secondary lymphatic follicle. It is composed of reticular cells and many dividing B lymphocytes.

11.3.7.2 Mantle

The mantle is a region of densely packed mature B cells present around the germinal center of the lymphoid follicle. This region also has many macrophages which protect the developing B cells and prevent them from interacting with any antigen escaping from adjacent blood vessels.

11.3.8 High Endothelial Cell Venules

High endothelial cell venules are present within the lymphatic organs which allow the movement of lymphocytes from the peripheral blood into the organs (homing). Also, mature (immunocompetent) lymphocytes return to the circulation and may reach certain organs/tissue passing through the wall of the sinuses. All vascular systems are lined by endothelial cells (flattened simple squamous epithelial cells). The heart, arteries, veins, lymphatic vessels are all lined by endothelial cells. Therefore, high endothelial venules are very special modifications from the regular vessels lining the epithelium.

11.4 Primary Lymphatic Organs

11.4.1 Bursa of Fabricius (Cloacal Bursa)

The cloaca is a combined space at the end of the digestive, urinary, and genital systems (for more information see Chapter 4, Digestive System). The cloaca has three compartments, proctodeum, urodeum, and coprodeum. The bursa of Fabricius is a dorsal diverticulum of the cloacal proctodeum (Konig et al. 2016). The bursa starts as a solid mass projection to the outside and later the internal epithelial lining develops leaving a lumen within it. The bursa in chicken has a chestnut shape (Singh 2019). Its growth

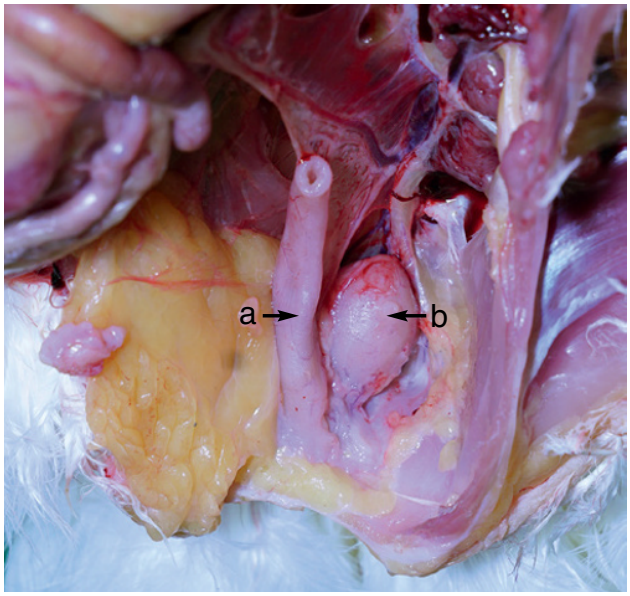

Figure 11.3 Bursa of Fabricius (b) of a young chicken (17 weeks) inside the celomic cavity associated with the colorectum (a).

coincides with the rapid bird body growth. The bursa reaches its full size at 6 weeks after hatching followed by regression (the beginning of the involution process like the thymus) which starts at 8–12 weeks according to Dyce et al. (2000) (Figure 11.3). However, Rose (1979) stated that it starts to involute at the time of maturation of the bird, and Ceriarco et al. (2003) stated that the bursa is heavily involuted by six to seven months of age. Histologically, it has a simple columnar to pseudostratified columnar epithelial lining like the cloaca. The epithelium has columnar, goblet, and periodic acid Schiff (PAS)-positive cells with granules, in addition to the undifferentiated cells at the base of the epithelium which have rounded nuclei and high regenerative capability to replace damaged epithelial cells.

It is surrounded by a thin layer of connective tissue capsule of main collagen and a few elastic fibers with trabecular extensions into the parenchyma to separate the organ by a thin layer of connective tissue into 12 longitudinal lobules or plicae (Hodges 1974, pp. 206–213). These plicae are like the villi projections of the intestine. Each lobule has a dense periphery called the cortex and a lightly stained area below the medulla. Both cortex and medulla are filled with epithelial-reticular cells (stellate) holding the lymphocytes and other cells (Figure 11.4). The stellate cells have desmosomes joining adjacent cells cytoplasmic processes with. The cortex and medulla are separated by tufts of blood capillaries. Dense cells, mainly small lymphocytes (B cells), populate the cortex resulting in a darkly stained region at the periphery of each fold (plica). Few T cells are present along with plasma cells in both the cortex and the medulla. Tunica muscularis is described to be inner circular and outer longitudinal with few instances of a third layer present as a second longitudinal layer (Hodges 1974, pp. 206–216).

The bursa is highly vascularized and innervated. The blood vessels enter the bursa through the capsule and branch inside the plica. The blood supply of each plica is independent of the adjacent one. The blood bursal barrier may be present in the medulla but not the cortex (Hodges 1974, pp. 206–2116). Sympathetic autonomic innervation to the bursa of Fabricius is from the hypogastric nerves which is a continuation of the aortic plexus. The parasympathetic portion originates from the spinal nerves 30–33 segments (Freedman and Sturkie 1963).

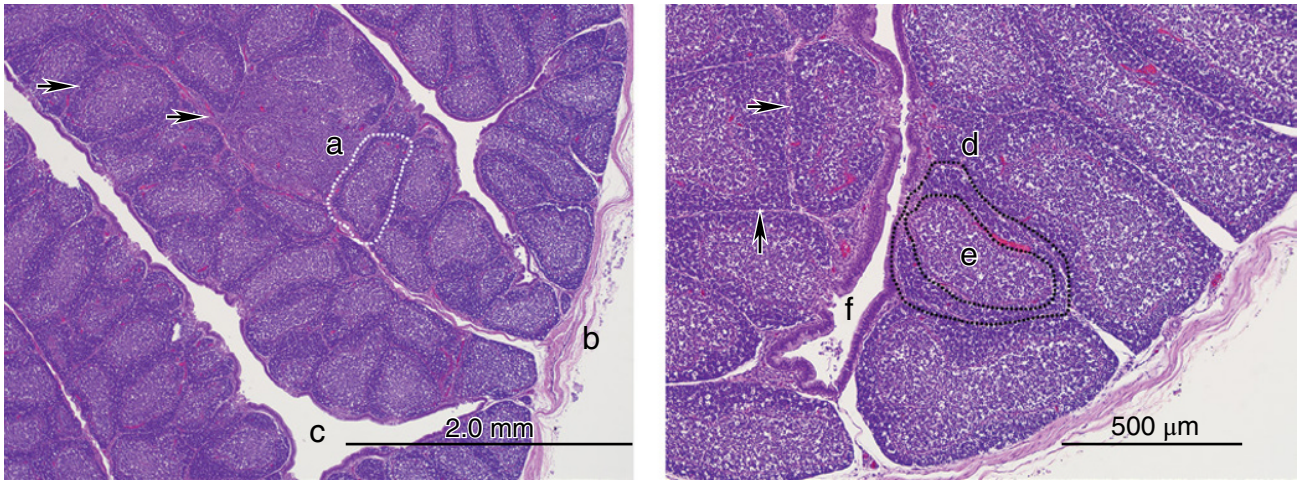

Figure 11.4 Bursa of Fabricius, H&E stain. Follicle filled with lymphocytes (a), connective tissue capsule (b), bursal lumen (c), follicular cortex (d), follicular medulla (e), luminal epithelium (simple columnar) (f), and follicular septae (arrows).

The bursa is the site where B lymphocytes attain their immunocompetent capabilities and function in defense mechanisms. Therefore, removal of the bursa in a one-day-old chick will fail in the full development of the B lymphocytes, while removing it from adult chicken may not affect the activity of the immune system. The bursa has a remarkable capacity for transcytosis of macromolecules at a modified epithelium which overlies the bursal nodules including antigens from the lumen into the lymphatic bursal nodules (Eurell and Frappier 2006). In a one-day-old chick, the bursa connects with the proctodeum where it developed from and continued to open into it. The bursa position at the exit of secretory and excretory materials may expose it to microorganisms descending from the chicken's different systems or ascending from outside through the cloacal drinking.

Scattered lymphoid tissue in the form of organized and non-organized lymphoid follicles is seen very close to the mucosal junction of the cloaca with the vent epithelium. These scattered lymphatic tissues play a role in the defense mechanism when cloacal drinking happens as microorganisms may enter through the vent cleft into the cloaca in addition to the microorganisms arriving with the ingesta from the digestive system.

11.4.2 Thymus

Bird thymus originates from the third, fourth, and fifth pharyngeal pouches. The primitive cells migrate distally to form a solid mass of cells of the future thymus. They are completely separated from the pharyngeal endodermal cells. Mesenchymal cells migrate to the thymus from the surrounding head mesenchyme and arrive through the blood vessels penetrating the thymic tissue. Head mesenchymal cells form the stroma except for the epithelial-reticular component which is endodermal in origin. These cells produce the meshwork or the reticulum within the thymus. The thymus migrates superficially along the cervical region close to the external jugular vein and vagus nerve to reach the entrance of the thoracic portion of the celomic cavity. It is usually represented by six to eight lobes completely separated from each other (Singh 2019) (Figure 11.5). One should be careful when describing the thymus location from the upper cervical to the first few thoracic vertebrae because it continues to migrate caudally and distally with age. Therefore, it will be higher in the cervical region at a very young age and lower with advancing age.

The thymus is covered by a thin connective tissue capsule surrounded by white adipose tissue mainly of collagen with few elastic fibers (Figure 11.5). This capsule sends bundles of connective tissue into the parenchyma of the

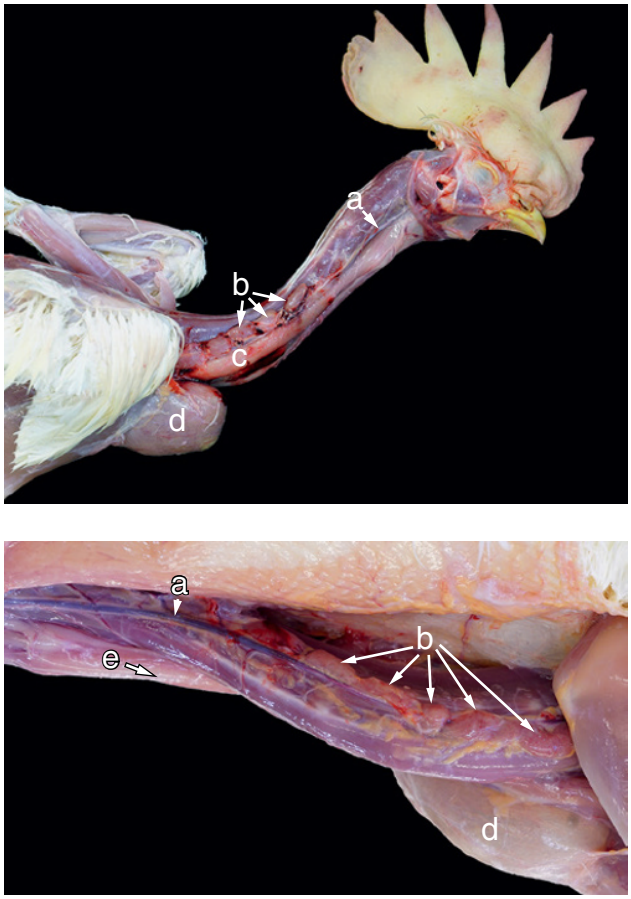

Figure 11.5 Chicken thymus. Right lateral (superior) and left lateral (inferior) views of the cervical region of the chicken showing the external jugular vein (a), thymic lobes distributed on the caudal cervical region (b), esophagus (c), crop (d), and trachea (e).

organ to separate the gross anatomical lobe into smaller lobules. The outer portion of the thymus is organized in highly populated cells outside of each lobe which forms the cortex. The cortex has the highest number of densely packed lymphocytes (thymocytes). The remaining inner portion of each lobe is less populated with cells that form the medulla (Figure 11.6). Thymus in birds reaches its maximum size (full size) two to three months after hatching and starts to involute after that. Thymic tissue is replaced by connective tissue cells and adipocytes. During thymic tissue development and proliferation, T lymphocytes arrive from the bone marrow to attain their immunocompetent potential. Once T lymphocytes attain their potential, they continue to clone and flourish regardless of the stage of the thymus involution. However, if the thymus is removed in a one-day-old chick, there will be a sharp decrease in the number of immunocompetent T cells to contribute to the defense mechanism of the animal body (Panigraphi et al. 1971).

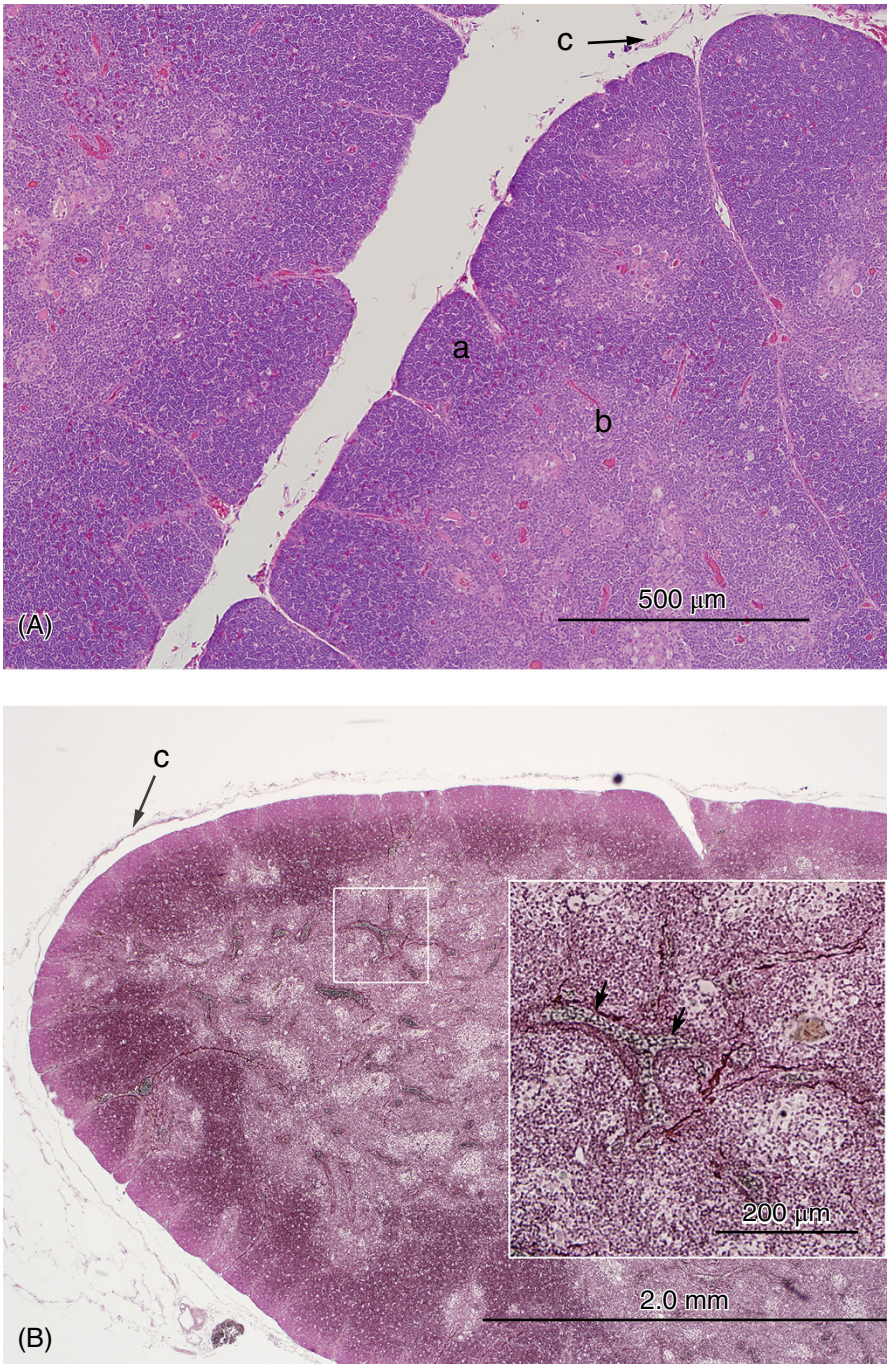

Figure 11.6 Adult chicken thymus. (A) H&E stain showing cortex (a) and medulla (b). The thymus has a very thin capsule (c). (B) Reticular stain. Notice the dark lines predominantly within the medulla in the insert (arrows).

Immature lymphocytes colonize the cortical regions of the thymus. Mature cells enter the medulla after the development of the receptors on their cell membrane. Lymphocytes within the thymus are called thymocytes. Dendritic cells are present to regulate and execute the negative selection of T cells. Hassall's corpuscles (thymic corpuscles or bodies) were reported to be present in the chicken thymus (Bódi et al. 2015). Hassall's corpuscles have degenerated epithelial-reticular cells arranged in a concentric manner present within the medulla, not in the cortex with unknown function. Thymic cortical epithelial cells are involved in the clonal selection of T-lymphocyte. Medullary epithelial cells are involved in the clonal deletion of potentially auto-reactive T lymphocyte cells.

A multicellular complex is identified in the chicken thymus which is present in the subcortical region of the thymus. These cells are described as antigen-presenting cells and given the name thymic nurse cells (TNC). Thymic epithelial cells express MHC on their cell membrane which, in turn, exposes the thymocytes to it (Silva and Gallardo 2020). The TNC provides a microenvironment for positive selection of thymocytes by engulfing them, exposing them to the MHC expressed by the thymic epithelial cells, and dictating the time of the release of thymocytes (Ricker et al. 1995).

11.4.2.1 Blood Thymic Barrier in Chicken

The blood thymus barrier has not been demonstrated in the chicken thymus. Raviola and Karnovsky (1972) stated that the barrier exists only in the cortex for an antigen-free microenvironment for thymocyte maturation to take place. The barrier is formed by the endothelial cells of the blood vessels and their basal laminae and the epithelial cells and their basal laminae. No explanation of why this difference between the cortex and medulla was found in the available literature. Most probably to prevent interaction between antigens from the blood vessels with the developing T lymphocytes. Macrophages are also present to stop the risk of an autoimmune reaction.

11.5 Secondary Lymphatic Organs

11.5.1 Spleen

The mammalian spleen acts as a blood reservoir, but the avian small size spleen does not perform this function. The spleen starts as a blood-forming organ at an early stage of embryonic development, though it loses this function and keeps the immune one. Removal of the spleen in chicken results in lower Ab production (Aitken 1973). The spleen does not function as a hemopoietic organ during early development or storage of red blood cells after hatching. The main immune function of the spleen starts after the development and maturity of the red and white pulps. This immune response ability will be specific for blood-borne antigens. The presence of many macrophages within the parenchyma of the spleen acts to remove foreign materials (molecules) escaping from the circulating blood. The bird spleen still removes non-viable red blood cells; therefore, hemosiderin pigment (brownish colored) may be detected under the microscope within the spleen parenchyma. The antigen that reaches the spleen will be taken by the antigen-presenting cell which then interacts with T helper lymphocyte. T lymphocytes pass it to B cells which differentiate into plasma cells and produce immunoglobulin.

The spleen in a fully developed bird is rounded in shape and located dorsal and to the left of the proventriculus. It is surrounded by a connective tissue capsule with a few smooth muscle cells (Figure 11.7). It is covered from the outside by a glistening membrane which is the visceral peritoneum. The peritoneum is a simple flatted mesothelial squamous in shape resting on a basement membrane. No clear separation of cortex and medulla in the bird spleen like that of mammals. Though the parenchyma may be described as having white and red pulps. White pulp is arranged like a lymphoid nodule (follicle) from a lymph node. The red pulp is dilated venous sinuses of uncircumscribed boundaries filled with blood, mainly red blood cells, thus the name sinuses is used (Figure 11.8). Modified fibroblasts from the meshwork of the spleen (reticular cells) produce an extracellular matrix. The matrix reticular

Figure 11.7 Chicken spleen. Left liver lobe (a), spleen (b), gallbladder (c), and right liver lobe (d), observe the surface of the spleen covered with a thin smooth glistening membrane which is the visceral peritoneum (e).

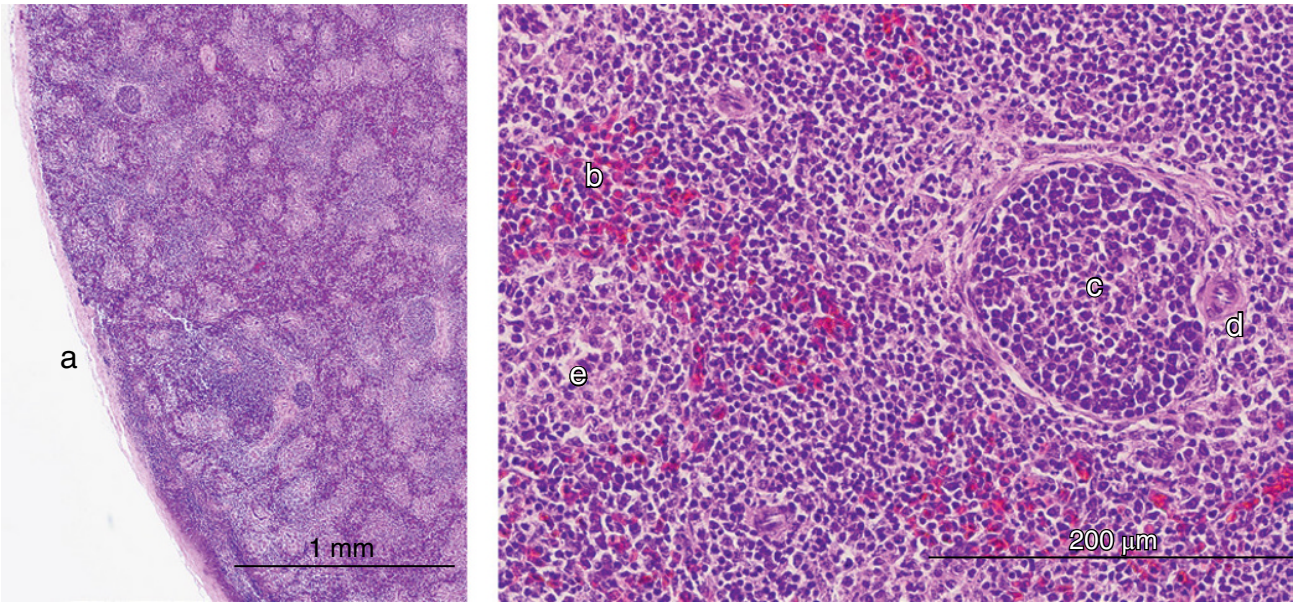

Figure 11.8 Chicken spleen, H&E stain. Capsule (a), red pulp (b), white pulp (c), and central arteriole (d). Notice that not all white pulps (lymphatic follicles) have connective tissue capsule around them (e).

fibers plays a role in the isolation of T, B, and dendritic cells. Dendritic cells phagocytose foreign substances and occasionally function as an antigen-presenting cell. Generally, germinal centers appear in the chick spleen for the first time at about four to five weeks of age. Three lymphoid tissues were described which consist of periarterial lymphatic sheaths (PALS), periellipsoidal lymphoid tissues (PELT), and germinal centers (Fukuta et al. 1980). The perisinusoidal lymphoid tissue corresponds to the marginal zone in mammals. White pulp has PALS and PELT (Jeurissen 1991; Zhang et al. 2015). These researchers also postulated that the blood spleen barrier is in the ellipsoid and outer compartment of the PELS. High endothelial vein-like vessels were identified as the site of the lymphocytes homing in the chicken spleen.

It has a germinal center when it is exposed to an antigen and the lymphocytes clone themselves within the white pulp. There is a central arteriole which most of the time after full development of the spleen migrates to the periphery of the white pulp. This arteriole is absent in the lymphoid follicle of the regular lymph node in other species.

It has been reported that considerable reduction in the white pulp in chicken takes place between six months to two years of age. The distinct separation between the germinal center and the mantle areas disappears. However, the central arteriole still has several lymphocyte cell layers around it (Hoffmann-Fezer et al. 1977).

11.5.1.1 Spleen Blood Supply

The splenic artery arises from the right branch of the celiac artery. It extends between the right lobe of the liver and the spleen where it provides cranial and caudal arteries to the spleen. In a few exceptions, a splenic artery may arise at the bifurcation of the celiac artery (Fukuta et al. 1976). The splenic artery branches into smaller arteries, trabecular followed by arterioles to reach the capillaries. These terminal vessels were described as penciller capillaries. They are freely open into the red pulp cords where they enter dilated sinuses between their endothelial cells (Nagy et al. 2004). Trabecular arteries continue as central arterioles within the white pulps, and they are enveloped by PALS. The chicken spleen is rich in ensheathed capillaries compared to the central arterioles. The splenic arterioles are described to be surrounded by venous sinuses and periellipsoidal capillaries (Hoffmann-Fezer et al. 1977).

The central arterioles present within the white pulp are surrounded by T lymphocytes. The germinal centers are usually found close to the central arterioles. Though, some arterioles are found at the periphery of the white pulp. These lymphocytes are located close to the tunica media of the arteriole and called periarterial lymphatic sheath (PALS). Between the periarterial and periellipsoidal white pulps (lymphoid tissue) which surround the penicillate capillaries, PALS have less pattern in the red pulp because they have scarce reticular fibers. Small lymphocytes are present around the arterioles, while both T and B lymphocytes are present in less number around the small venous sinuses. Less number of lymphocytes are present among the sinuses and the cords of the red pulp.

Blood Spleen Barrier

To understand the barriers in the lymphatic system and any other system, one must understand the junctional

complexes and their type between adjacent cells of the surface epithelium, endothelium, basal lamina type, and size of secretory products produced.

11.5.2 Cecal Tonsils

The cecal tonsils are two small enlargements at the beginning of the two ceca in chicken on both sides of the ileum (Figure 11.9). Diffuse and nodular lymphoid tissues are present in the cecal tonsils which are located at the proximal end of the cecal pouches. Many lymphocytes appear in this specific region of the organ during the first week of age. The full development of the tonsils depends on the presence of the microflora (Oláh and Vervelde 2008). Though germinal centers appear at week 2 of age. It is reported that chicken raised in a germ-free environment will not develop germinal centers. These centers increased in number as the chick aged and that is due to the possibility of exposure to germs or antigens. If the microflora is destroyed or not present, the cecal tonsils (lymphoid tissues) decrease in number even after fully developed. The mucosa of the ceca in chicken is like the rest of the intestinal tract. They have villi covered by simple columnar epithelium with goblet cells. Tonsils aka lymphoid aggregates are present within the lamina propria and the submucosa. More goblet cells and lower villi height were reported in the areas where the tonsils were present (Rezaian and Hamedi 2007).

The presence of lymphoepithelial cells, subepithelial zone, germinal centers, and interfollicular areas are commonly present within the cecal tonsils (Figure 11.10). Many small lymphocytes, developing plasma cells, and macrophages are always present.

Foreign materials may reach the bursa of Fabricius and or the cecal tonsils through the process of cloacal drinking where the cloacal lips move inward and with the antiperistalsis capability of the intestine the materials reach the cecal tonsils. Antiperistalsis is common in birds and thus urine may flow back from the proctodeum to the colorectum and reach the cecum. Therefore, cecal tonsils are continuously exposed to microorganisms from outside the body and from within the body arriving at the ceca with the digested food or through the blood (Tizard 1979).

The buildup of pressure on the wall of the ceca results in stimulating the autonomic nervous system signaling the smooth muscle cells in the wall of the ceca to contract to empty its content. The longer the food stays within the lumen of the ceca, the more chances are that foreign particles interact with the cecal tonsils and initiate an immune response. These interactions stimulate the proliferation of cells within the germinal centers and lead to the production of plasma cells and consequently production of antibodies which acts locally as a defense mechanism. Like other lymphoid tissue, the cecal tonsils regress with age.

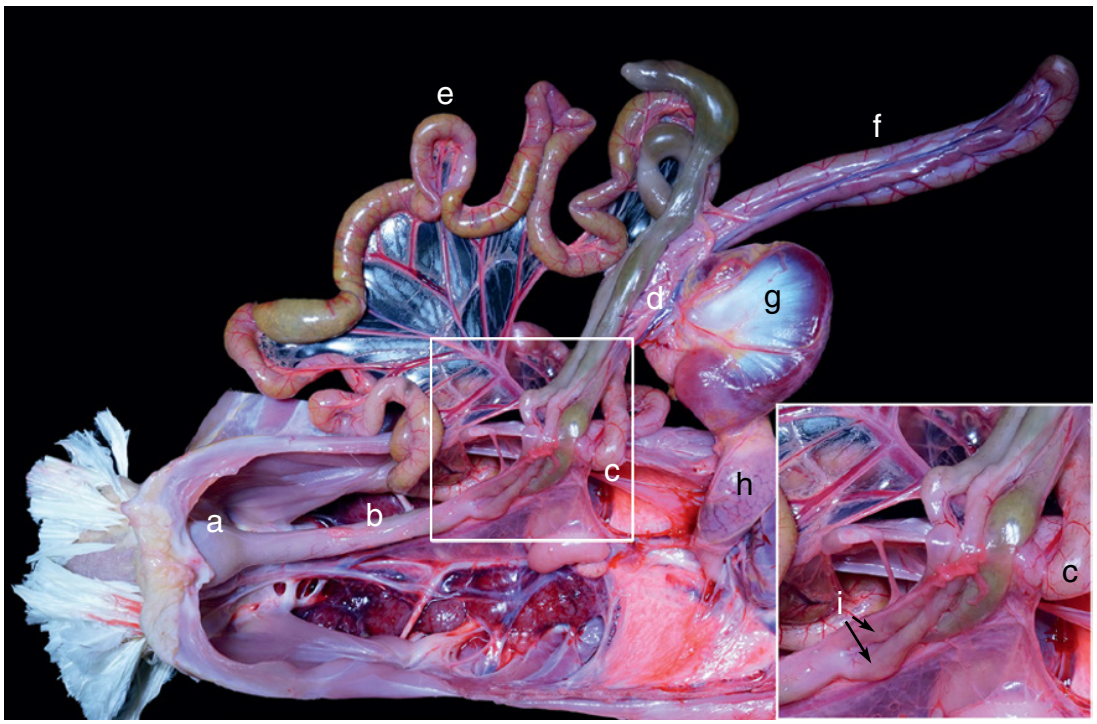

Figure 11.9 Celomic cavity with digestive organs. Cloaca (a), colorectum (b), ileum (c), left cecum (d), jejunum (e), duodenum (f), ventriculus (g), proventriculus (h), and cecal tonsils (i). Inset: magnification of the ileocecocolic junction.

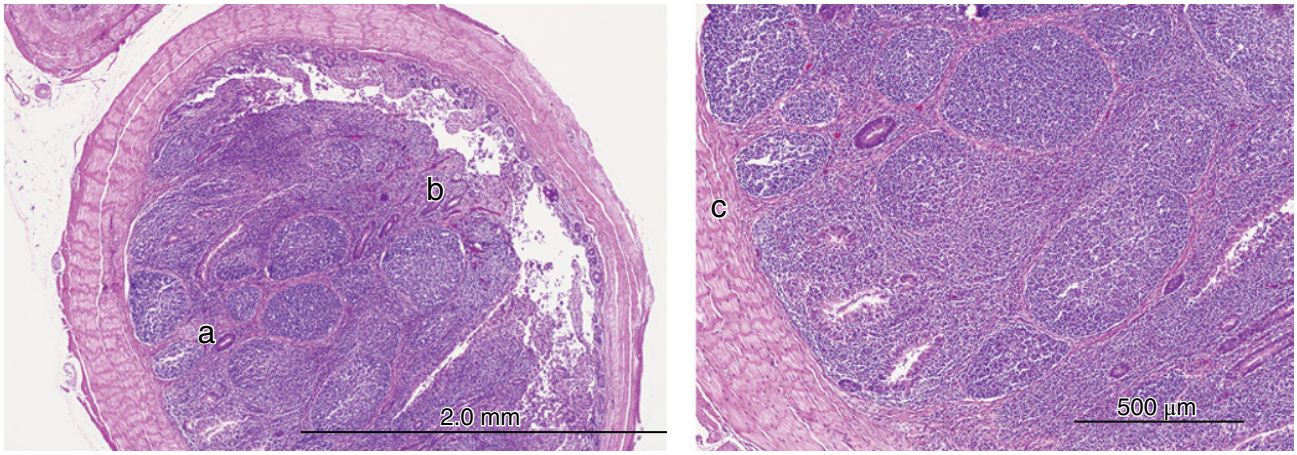

Figure 11.10 Cecal tonsil of adult chicken, H&E stain. Notice the lymphatic nodules (a) filled the submucosa (c) and the lamina propria (b).

11.5.3 Gut-Associated Lymphoid Tissue

GALT is an aggregate of intramural lymphoid tissue usually present within the wall of the gastrointestinal system in the normal physiological condition and their concentration varies from one region to another. Their presence usually is close to the natural orifices and wherever there is some potentials of microorganisms penetrating the wall and changing the environment of the region. These lymphoid tissues may be nodular (follicular) or diffuse. Nodular implies that they form a regular lymphatic nodule either surrounded by connective tissue or just a nodular form without a capsule. These nodules are very similar to the ones present inside the lymph nodes in mammals and the white pulp of the spleen. They could be primary when they are not exposed to an antigen, and secondary when they are exposed to a specific antigen resulting in the formation of a germinal center and a mantle. B lymphocytes are concentrated within the germinal center of the nodule, while T lymphocytes are present in between the nodules (Oláh et al. 2003). It is reported that GALT is present along the entire digestive tract in chickens. Oláh et al. (2003) described the number of esophageal tonsils in white leghorn chicken at the junction of the esophagus and the proventriculus. The tonsils match in number with the number of folds (crypts) at that junction. Those at the esophageal portion are covered by stratified squamous epithelium and limited to the lamina propria only, while those at the beginning of the proventriculus are covered by simple columnar epithelium. Their location at the distal portion of the esophagus and the beginning of the proventriculus expose them to food particles, microorganisms, and other foreign materials ingested by the chicken.

Lymphocytes normally penetrate the surface epithelium for surveillance and interact with any antigen escaped from within the gut lumen (Figure 11.11). Some researchers stated that an increased number of these lymphocytes within any epithelium will change the epithelium name into lymph-epithelium. However, GALT under normal physiological conditions is present within certain layers in the tubular organs like lamina propria or extends into the tela (tunica) submucosa. With any pathological changes anywhere in the body, lymphocytes in large numbers may be called to the site especially after a certain period (acute state) to interact with the macrophage and may eventually infiltrate the surface epithelium. M cells are typically found in the Peyer's patches of the chicken and mammal ileum (Befus et al. 1980; Burns and Maxwell 1986) and within the cecal tonsils (Jeurissen et al. 1999; Kitagawa et al. 2000). The M cells have special receptors for specific antigens on their apical cell membrane and they interact with the ingesta and usually sample or phagocytose microorganisms or other intestinal antigens. It transports the antigens to the underlying dendritic cells at the outer surface of the GALT to initiate an immune response. The antigens within the lumen of the intestine are picked by M cells, delivered to macrophages which process the epitopes, and presented it to T cell then to B cell which differentiates into plasma cell to produce Ig (antibody) either locally or moves to the closest lymphatic structure. The main immunoglobulin produced is IgA. It is reported to be the central component of intestinal homeostasis (Pietrzak et al. 2020). This is a local response by the GALT (Peyer's patches) to an antigen invading part of the gastrointestinal tract. This kind of movement is encouraged by the process of absorption throughout the entire length of most of the gastrointestinal tract. Therefore, there must be a balance to keep the

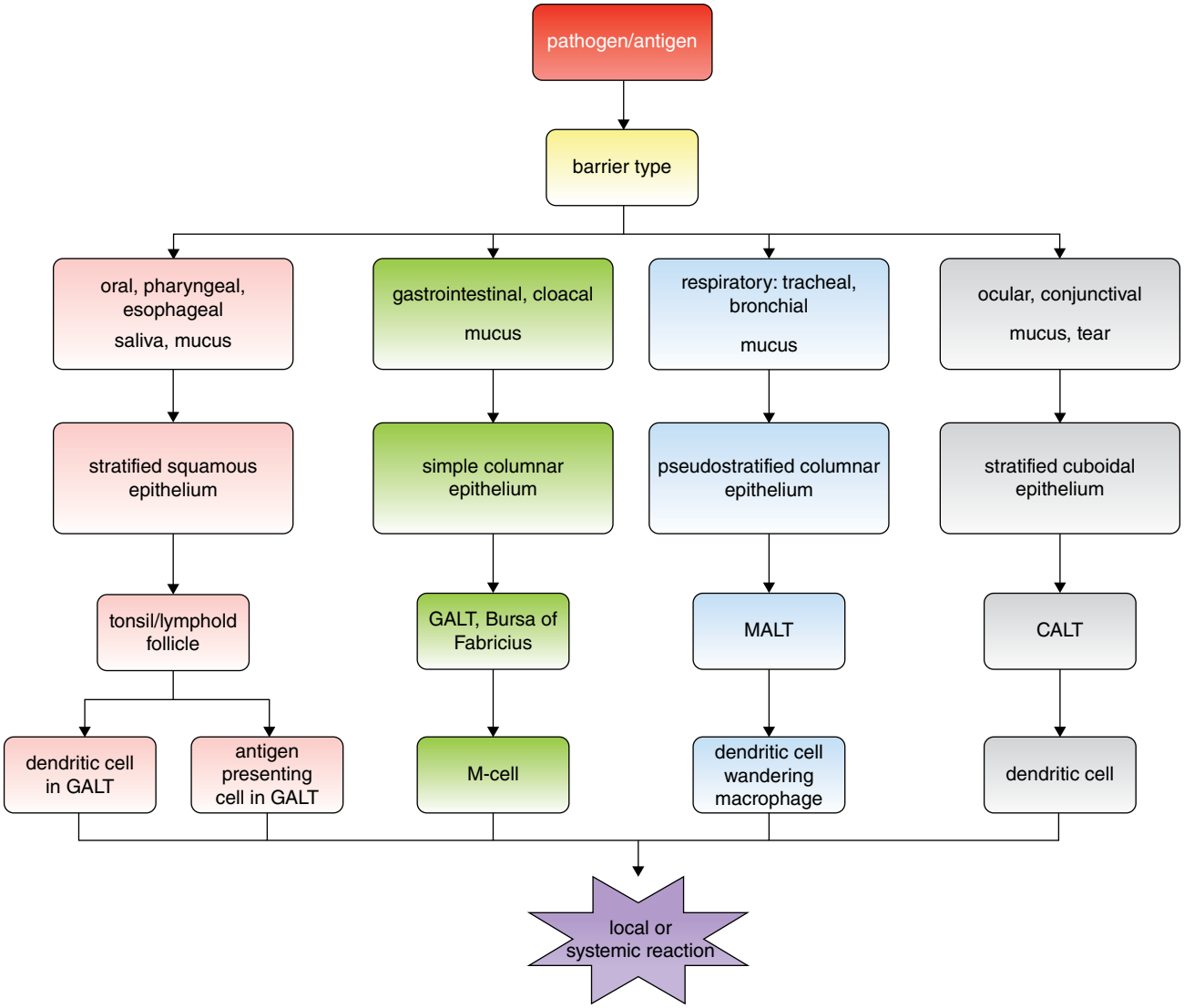

Figure 11.11 Description of the lymphatic system reaction toward pathogens and the barriers represented by different structures depending on the body region of exposure. GALT, gut associated lymphoid tissue; MALT, mucosa associated lymphoid tissue; CALT, conjunctival associated lymphoid tissue.

environment of the wall of the intestine sterile to a certain extent by preventing the commensal microorganisms from damaging the normal physiological homeostasis.

11.5.3.1 Peyer's Patches

Peyer's patches are an aggregate of many lymphoid tissues in the wall of the ileum within the small intestine. The ileum is lined with simple columnar epithelium resting on a basement membrane with a thin layer of connective tissue. The epithelium is exposed to the food particles within the lumen of the intestine along with the microflora which finds a favorable environment to survive under normal physiological conditions and may overcome the barrier and the inflammatory process to reach the lamina propria (Shi and Walker 2015, pp. 9–29). The lamina propria, which is immediately below the epithelium, is rich in blood and lymphatic vessels, especially at the tip of the villi where the transfer of large fat (triglycerides) molecules occurs from the lumen to the dilated lymphatic vessel (lacteal). GALT acts to keep the equilibrium between the two compartments (intestinal lumen and the intramural part where the lamina propria is located. Peyer's patches function in the same way as any other lymphatic tissue/organ. Within the lamina propria, there are many immune and non-immune cells and molecules moving in and out of the different tunics of the intestine. Many small lymphocytes, plasma cells at different stages of development, as well as macrophages are present in the Peyer's patches. Foreign

particles from within the lumen of the intestine pass through the intestinal barrier to reach the M cells which hand it over to the antigen-presenting cells.

11.5.3.2 Meckel's Diverticulum

Meckel's diverticulum is the remnant of the communication between the midgut and the yolk sac. It facilitates the transfer of nutrients from the yolk sac to the developing intestine of the chick. This structure regresses from its connections with the yolk sac and leaves a small projection in the middle of the jejunum. The muscular wall of this structure is thin compared to the wall of the jejunum (almost half of the size of the muscular wall of the jejunum). It is filled with lymphoid tissue with large number of macrophages, small lymphocytes, and plasma cells. These cells fill the lamina propria around the glandular tissue to be close to the surface epithelium of the jejunum. This way, it has access to the food particles and any foreign materials within the lumen to process and take to the lymphatic system.

11.5.4 Harderian Gland

The Harder or Harderian gland of fowl is classified as compound tubular-acinar type I (Figure 11.12), which means that a single cell type lines the epithelium of each glandular lobule (Burns 1992). This gland presents also abundant plasma cells, different sizes of lymphocytes, mast cells, macrophages, and melanin pigments that were present in the interlobular tissue (Verma et al. 2019). These plasma cells decrease in number with age (Survashe 1976). Any exogenous antigen may enter the chicken eye by using the excretory duct of the gland to stimulate a local immunologic response (Burns 1992). Immunological response in the chicken Harderian gland has been documented by Albini et al. (1974) (Figure 11.12).

11.5.5 Mucosal Associated Lymphoid Tissue

MALTs are scattered lymphoid tissue under epithelium in different tubular systems other than the gastrointestinal. Oral and nasal mucosae are excellent examples and common sites for the entrance of microorganisms. For any natural orifices or site of the possible entry of microorganism, there will be a barrier.

11.5.5.1 Conjunctival Associated Lymphoid Tissue, Conjunctival Lymph Nodules

Conjunctival associated lymphoid tissue in the form of nodules were detected in the lower eyelid in chicken and fewer in the upper eyelid at an early age after hatching with the heterogeneous cellular composition of lymphocytes, lymphoblasts, and macrophages. Plasma cells are observed only in follicles with germinal centers. The epithelium over the nodule is composed of stratified squamous cells with a discontinuous basement membrane. The absence of goblet cells and intraepithelial lymphocytes results in the formation of what is called a lymph-epithelium. Evident vessels at the base of the lymphoid nodules have high endothelial cells (Fix and Arp 1991). Antigens may easily access the conjunctiva and become in contact with the surface epithelial cells which deliver it to the underlying lymphoid follicles.

11.5.5.2 Nasal Associated Lymphoid Tissue

Nasal-associated lymphoid tissue has been described in humans as well as rodents and chickens. Because of the intranasal vaccine delivery in birds, it gained lots of attention from researchers. Kang et al. (2013) described them in chicken to be located on the bottom of the nasal septum and on both sides of the choanal cleft. They mainly consist of secondary lymphoid follicles. Many lymphocytes were distributed under the mucosa. Diffuse lymphoid tissue is reported in the epithelium of the inferior nasal meatus and middle nasal concha as well as the wall of the nasal cavity. Lymphoid tissues have been demonstrated in the oculonasal region, nasolacrimal duct, lateral nasal glands, and ducts (Bang and Bang 1968).

11.5.5.3 Trachea and Lung Lymphoid Tissue, Mucosal Associated Lymphoid Tissue

The movement of air in chicken is unidirectional and thus there is a higher possibility of mucus movement using the mucociliary escalator. The movement of the mucus starts at the bronchioles up to the trachea and carries the small particles out of the respiratory tract. However, larger particles within the parabronchi are engulfed by the alveolar macrophages.

The upper and lower respiratory systems may differ in the amount of lymphoid tissue distribution. Besides, it is reported that lymphoid tissue is well organized and diffused tissues are present in the chicken lung (Bienenstock et al. 1973; Van Alstine and Arp 1988). No M cell was observed in the chicken MALT from this anatomical region (Fagerland and Arp 1993).

11.5.5.4 Bronchiole-Associated Lymphoid Tissue

Bronchiole-associated lymphoid tissue is present at the junctions of the primary bronchus and the caudal secondary bronchi. These lymphoid tissues are constantly present to compensate for the absence of lymph nodes. The respiratory system in chicken has few resident macrophages compared to mammals and they are absent from the air capillaries but present in atria and infundibulum (Maina 1982). Responses to antigen exposure may take place locally or the antigen is transported to the spleen.

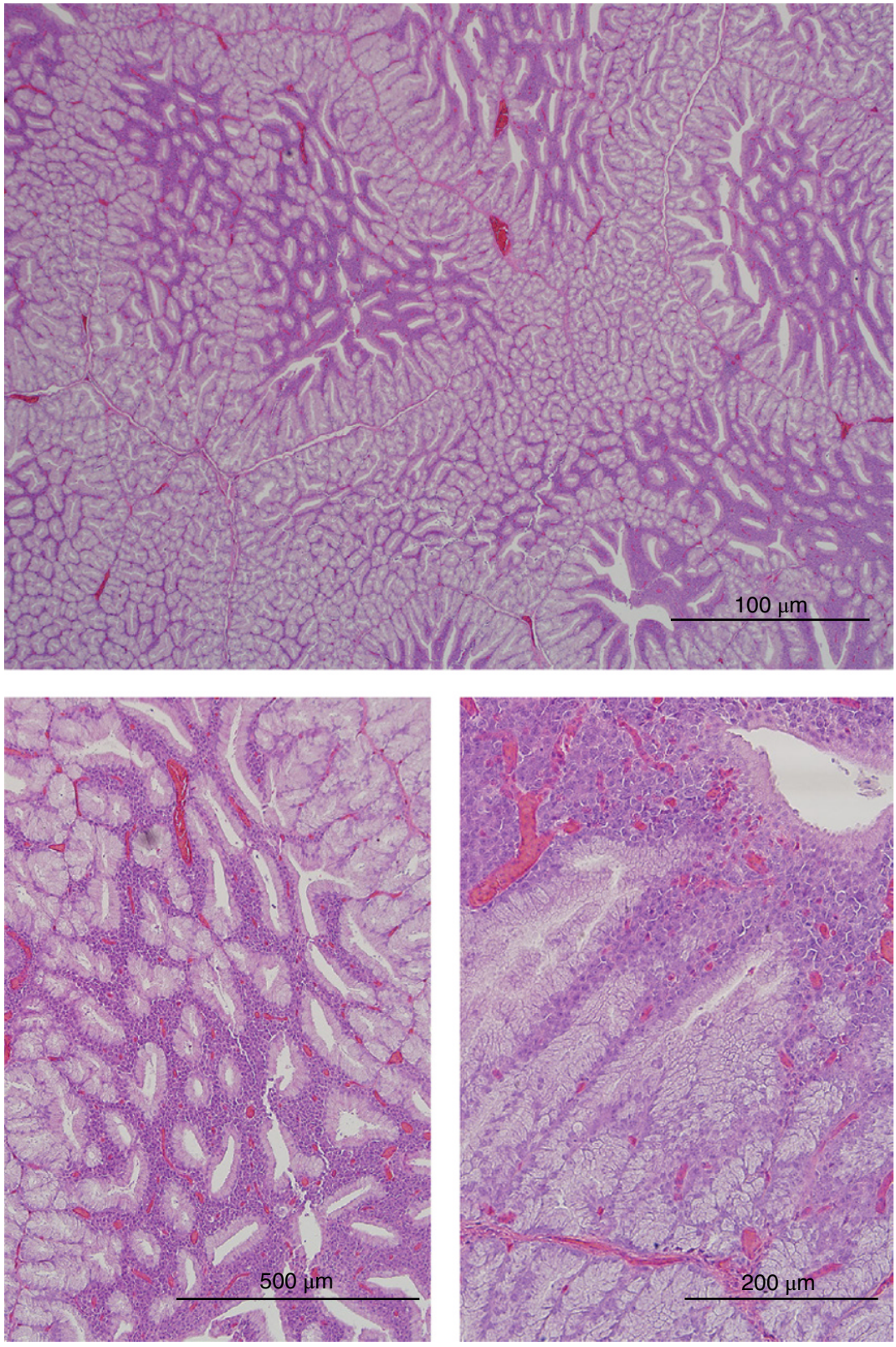

Figure 11.12 Harder gland section. H&E stain sections. Notice the secretory portion is stained light in color while the darker regions are filled with lymphocytes and plasma cells.

11.5.6 Lymph Nodes

Lymph nodes are generally absent in chickens; however, they were described in Geese and Swan (Nochi et al. 2018; Singh 2019).

11.6 Maternal Transfer of Immunity

After hatching, the chick has some immunity for protection against certain pathogens or diseases depending on the health status of the hen and or the types as well as methods of vaccination protocol followed. Immunoglobulin is the main molecule that acts to transfer immunity from the hen to the egg. The evidence in the available literature indicated that immunoglobulin (Ig) is deposited on the surface epithelium of the oviduct and transferred as IgG into the yolk sac. The yolk sac is highly vascularized, and IgG is transferred by receptor-mediated endocytosis across the yolk sac epithelium (Linden and Roth 1978). One needs to take into consideration the importance of maternal immunity (antibodies) which persists for several weeks after hatching but may also interfere with the active vaccination of newly hatched chicks.

The developing chick acquires maternal immunity from the yolk sac. This immunity appears in the fetal circulation (Davies et al. 1995; Derache et al. 2009). Remnants of yolk sac remain after hatching for a day or so and absorption of IgG continues to take place. Also, albumin from the blood diffuses into the amniotic fluid, and therefore, the chick swallows it thus acquiring IgM and IgA. A newly hatched chick has IgG in circulation and IgA in the intestine (Swayne 2013).

11.7 Lymphatic Vessels

Lymphatic vessels like any other blood vessel inside the animal body are lined with endothelium. It is simple squamous epithelial cells resting on a basement membrane and a thin layer of connective tissue which represents the tunica adventitia. These vessels frequently have bicuspid valves, especially at the branching sites or when joining a large vessel. The movement of lymph fluid within these vessels depends on the presence of smooth muscles within the wall of large vessels, contraction of skeletal muscles, and effect of adjacent arterial pulsation. These are in addition to the negative pressure within the thoracic portion of the coelomic cavity which draws the lymph toward the heart. The lymphatic vessels are relatively larger in diameter and more irregular than their counterpart blood vessels. These facts are due to the thin wall of the lymphatic vessel wall to allow larger molecules from the interstitial tissue to pass through.

Lymph fluid is collected through the small lymphatic capillaries from the interstitial tissue almost all over the body with very few exceptions. The vessels that collect lymph from the interstitial tissue capillaries to deliver to a lymph node (when present in certain breeds) are called afferent vessels, while those which draw lymph from a lymph node or other organ are called efferent lymph vessels. In general, lymph fluid drains into an organ like the spleen, thymus, or the bursa of Fabricius or they join a larger lymphatic vessel. These larger lymphatic vessels represent an organ or a body system to form a larger vessel to open either directly into the heart or a vein larger than the heart. Therefore, all lymph fluid and cells return to the blood circulation after leaving the heart.

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12

Nervous System

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12.1 Introduction

The CNS of bird is composed of similar segments to that of mammals although several differences exist between birds and mammals including the absence of gyri and sulci. Therefore, the cortical surface area of the brain is much smaller in birds. Another characteristic feature is that there is no clear line of demarcation between the pons and medulla oblongata. A short extension of the olfactory bulb is obvious in birds, and the bulb is smaller than the mammalian counterpart. Other segments of the bird brain are missing such as pyramidal tracts, trapezoid bodies, and corpus callosum. Though the same number of cranial nerves (12 pairs) are present in avian and mammals.

The presence of the glycogen bodies (absent in mammals) at the caudal portion of the spinal cord is an additional unique difference compared to mammalian CNS. The glycogen bodies are metabolically inert and may function to support the process of myelin formation in the avian CNS (Benzo and De Gennaro 1983). The vertebral column is long in birds; therefore, more functions are assigned to the spinal cord compared to mammals.

12.2 Neuron

The neuron is the cell and basic functional unit of the nervous system, in both the CNS and the peripheral nervous system (PNS). The neuron consists of a soma (cell body), perikaryon (peri-around and karyon-nucleus), axon, and dendrites. It usually has one axon and either one or more dendrites depending on the type and function (connection) of the neuron. The neuron of chicken is remarkably similar to those of mammalians. Regular organelles are present within the neuronal cell body and the axoplasm. The shape of the cell body varies depending on the type of neurons and their function. The neuronal cell body contains Nissl's substances which are aggregates of polyribosomes scattered all over the cytoplasm or presented as clumps. This substance is absent along with other organelles at the origin or the axon (axon hillock). The neuronal axon is the thin unbranched projection/process of the neuron. It arises from the neuronal cell body at the axon hillock where the action potential initiates. Therefore, the axon hillock is a part of the soma (neuronal cell body) and the site of origin of an axon. This portion of the cell is devoid of organelles and has higher ion exchange compared to the rest of the cell to facilitate the initiation of action potential. This region fires before the rest of the neuronal cell body; thus, one can state that this region is the pacemaker of the neuron. The dendrites are short extensions of the neuronal cell body that convey impulses received from axons from other neurons to the soma. Within the CNS, many processes (axons and dendrites) plus glial cells are called neuropil in the absence of the neuronal cell body.

12.2.1 Type of Neurons

12.2.1.1 Unipolar Neuron

The unipolar neuron has one extension which immediately divides into two branches, one is the receptor branch and the other one is the effector part. Dorsal root ganglionic cells are good examples of this type of neurons. It is also called pseudo-unipolar neuron.

12.2.1.2 Bipolar Neuron

The bipolar neuron has two projections, usually one axon and one dendrite. The best examples of this type are sensory organ neurons (eye-vision and inner ear-auditory).

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12.2.1.3 Multipolar Neuron

The multipolar neuron has more than two projections with one axon and several dendrites. The best example is the motor neurons within the ventral horn of the spinal cord.

12.2.2 Synapse

A synapse is the gap between two neurons or between the neuron and an effector organ (muscle or gland). It consists of pre- and post-synaptic elements and a synaptic cleft. Each neuron ends in expansions (boutons or buttons) to terminate at, or close to, the synaptic cleft. These buttons carry vesicles to be released when an action potential initiated at the soma and propagated through the axon arrives to the button. It transmits the electrical impulse from one neuron to the next one by releasing a neurotransmitter (produced by the soma) to fill the gap (cleft), thus allowing the impulse to pass through.

12.2.2.1 Types of Synapses

The axo-somatic synapse is between an axon and the soma (neuronal cell body). Axo-axonal synapse is between one axon and another. Axo-dendritic synapse is between an axon and a dendrite or a spine of a dendrite.

12.3 Ganglion

Ganglion (pl. ganglia) is an aggregation of neuronal cell bodies outside CNS. Most of the ganglia observed grossly during dissection are autonomic sympathetic with very few exceptions of other modality like the distal sensory ganglion of the vagus (Wakley and Bower 1981) or the dorsal root ganglia.

12.3.1 Sensory

Sensory ganglia can be found in the dorsal root of the spinal nerves and the distal sensory ganglion of the vagaus nerve. The neurons within this kind of ganglion have a centrally located nuclei surrounded by satellite cells (Figure 12.1).

12.3.2 Autonomic

In the autonomic nervous system, sympathetic ganglia can be found outside the organs they supply like the cranial cervical, middle cervical, cervico-thoracic, celiac, cranial, and caudal mesenteric. They are usually closer to the spinal cord than the parasympathetic ganglia. The parasympathetic ganglia are close to or inside the organ wall they supply (called terminal ganglia). Good examples of terminal ganglia are the myenteric and submucosal

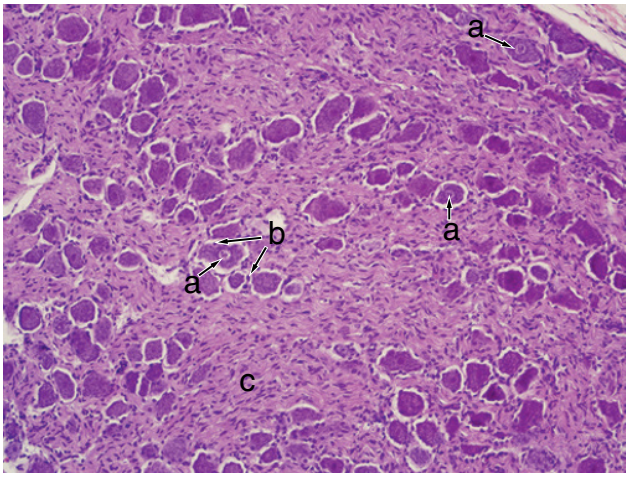

Figure 12.1 Histological section of a dorsal root ganglion. (a) Neuronal cell bodies with centrally located nuclei, (b) satellite cells surrounding the neuronal cell body, (c) axonal fibers. H&E stain.

within the entire gastro-intestinal and other tubular organs. The neurons of the autonomic ganglia are surrounded by connective tissue and the neuronal cell bodies have eccentrically located nuclei and surrounded by satellite cells (Figures 12.2–12.4).

12.4 Glial Cells (Neuroglia)

Glial cells are the cells other than the neuronal cell bodies within the CNS. They perform several functions depending on their type and location. Glial cells also release bioactive substances (Hanani and Spray 2020). Glial cells support, give structure, maintain ionic milieu to the function of the neurons, and help the process of healing in case of cell damage or removal of foreign debris. There are four types of glial cells within the CNS (astrocyte, microglia, oligodendrocyte, and ependymal). Two glial cells present outside the CNS are the Schwann and satellite cells.

12.4.1 Microglial Cell (Microgliocyte)

Microglial cells are mesodermal in origin, from the lineage of monocyte family (macrophage in tissue) and located in the bone marrow at the early stage of development. They can easily be distinguished by their small and elongated nucleus with irregular processes (pseudopodia). These cells can be found in all parts of the CNS, but they are the least abundant glial cells. They are also present close to capillaries; therefore, they form pericapillary satellite cells (Hodges 1974, p. 594). These cells act to remove foreign substances and debris from damaged neurons.

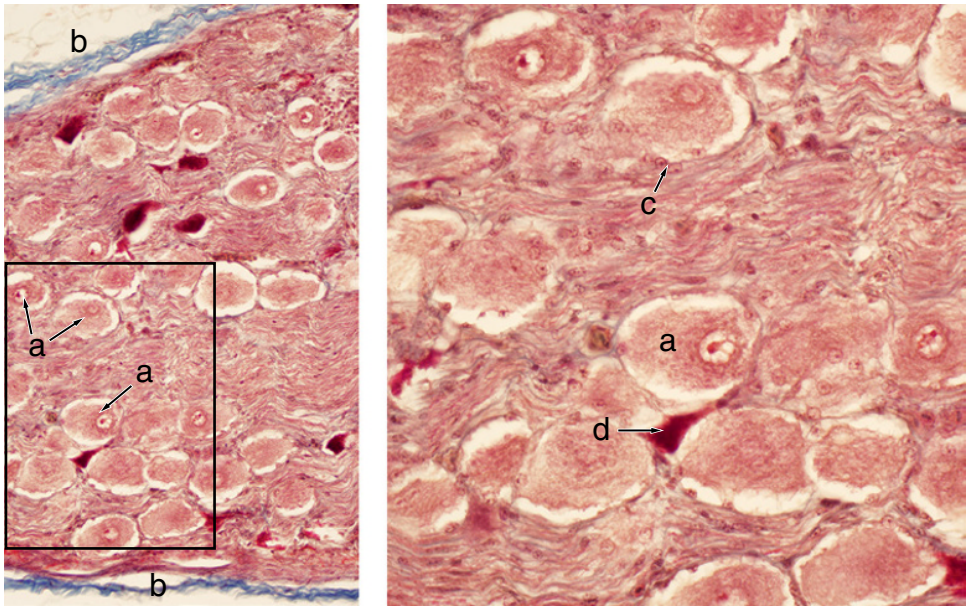

Figure 12.2 Autonomic ganglion of a chicken showing (a) neuronal cell bodies with eccentric nuclei, (b) connective tissue around the ganglion, (c) satellite cells, and (d) blood vessel. Trichrome stain.

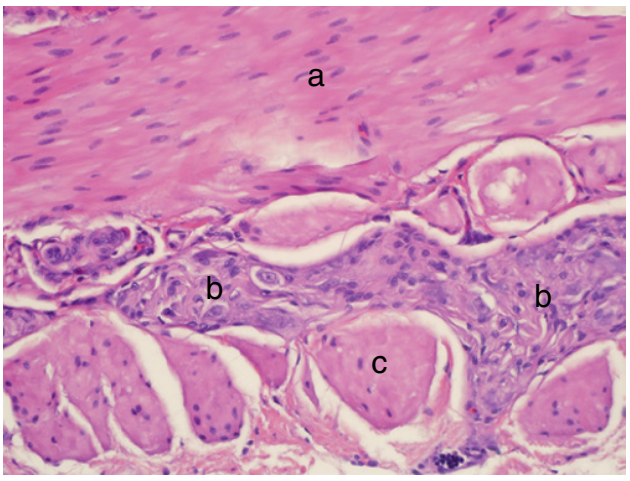

Figure 12.3 Myenteric ganglion in the wall of small intestine of chicken. (a) Inner circular smooth muscle layer, (b) myenteric ganglion with neuronal cell bodies, and (c) outer longitudinal smooth muscle layer. H&E stain.

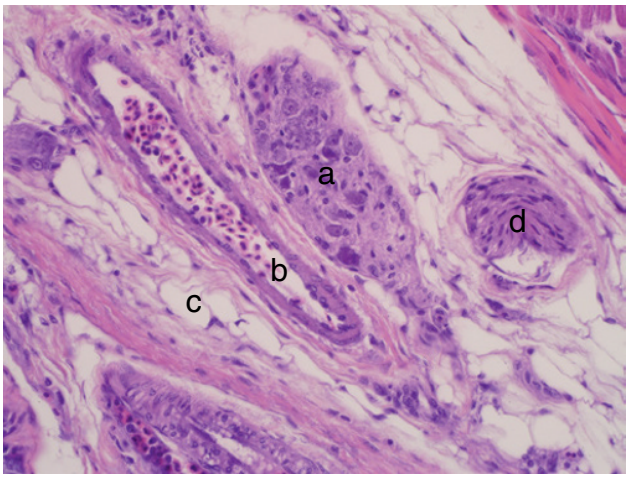

Figure 12.4 Submucosal ganglion within the wall of the upper digestive system (hard palate) showing (a) a ganglion with neuronal cell bodies, (b) vein, (c) white adipose tissue, and (d) nerve fibers. H&E stain.

12.4.2 Macroglial Cells

Macroglial cells are comprised of two types of astrocytes and oligodendrocytes.

12.4.2.1 Astrocyte

Astrocytes are the largest of the glial cells with rounded nuclei and irregular periphery due to the projections of processes away from the cell body. These processes may be primary and secondary and may appear at any level like the mammalian astrocytes (Hodges 1974, p. 591). Among the considerable number of functions described for the astrocytes are structural support, metabolic support that modulates electrical circuitry of the adjacent neurons, maintenance, and sensing of changes in the environment for the neurons. Recently, researchers showed that astrocytes activate CNS autonomic sympathetic control circuits after they detect low cerebral perfusion pressure and increase the systemic arterial blood pressure and heart rate (Marina et al. 2020).

12.4.2.1.1 Fibrous By name, the fibrous cell is star-shaped due to several processes. It is present within the CNS close to the neuronal cell body. Fibrous astrocyte is

present in white matter close to the blood vessels or the ependymal cells thus contributing to the blood brain barrier. Fibrous astrocytic end feet supports the ependymal cells to prevent passage of certain substances into the brain tissue because the latter lacks basement membrane.

12.4.2.1.2 Protoplasmic Protoplasmic astrocytes have thicker processes and are usually present within grey matter close to the neuronal cell bodies.

12.4.2.2 Oligodendrocyte

Oligodendrocytes have rounded cell bodies with short and thick extensions from the soma. The extensions give rise to long projections which elongate to wrap myelin sheath around several axons for insulation. Each oligodendrocyte wraps myelin around several axons. Most of their nuclei are round to oval. The relationship of oligodendrocytes to blood vessels was not well known but described to be close to small blood vessels (Stensaas and Stensaas 1968).

12.4.3 Ependymal Cell

Ependymal cells are a simple cuboidal to simple columnar epithelial cells lining the brain ventricles and the central canal of the spinal cord. At the level of the choroid plexuses within the brain ventricles, they interdigitate with a tuft of blood capillaries and pial cells (cells from the pia mater, inner layer of the meninges). The choroid plexuses produce the cerebrospinal fluid (CSF).

12.5 Peripheral Nervous System Cells

12.5.1 Schwann Cell (Lemmocyte, Neurolemmocyte)

Schwann cells are present only in the PNS to produce the myelin sheath insulation around the axons. Several Schwann cells are present around one single axon and each produces part of the myelin sheath. Through the insulation, they facilitate the propagation of the action potential along the axon to the target organ/tissue. They support the axon and phagocytize debris after axonal damage. All axons, whether myelinated or unmyelinated, are supported by Schwann cells which form a thin layer of plasmalemma around the axons in the unmyelinated while form a thick layer of myelin around the myelinated axons. All peripheral nerve axons are surrounded by three layers of connective tissue (endoneurium, perineurium, and epineurium) (Figure 12.5).

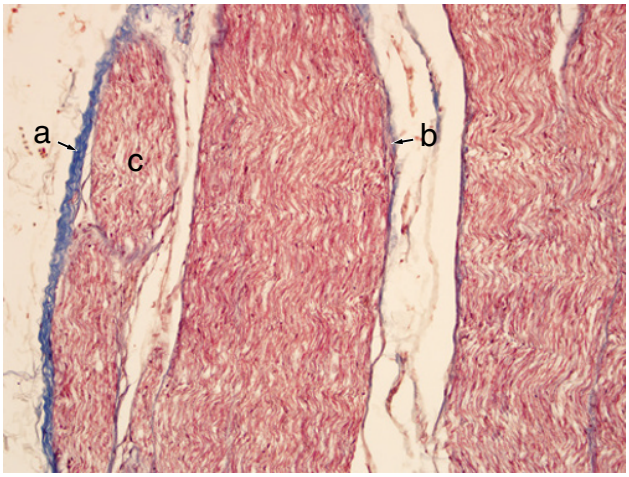

Figure 12.5 Longitudinal section of the ischiatic nerve of a chicken showing (a) epineurium, (b) perineurium, and (c) Schwann cells (darkly stained rounded nuclei). Trichrome stain.

12.5.2 Satellite Cell

Satellite cells are present within the ganglia. They are arranged around the periphery of the neuronal cell body. Their function is to control neuronal homeostasis and they have a bigger role after neuronal damage (Hanani and Spray 2020).

12.6 Meninges

The meninges are connective tissue membranes that surrounds the CNS, brain, and the spinal cord, in mammals and birds. In chicken, the outer and inner layers are described by Hodges (1974, pp. 589–590). The outer layer is fibrous connective tissue (dura mater) attached to the endosteum of the calvarium (skull cavity). The inner layer is the pia mater, and the extremely thin middle layer is the arachnoid. Since they are fused, sometimes they are referred to as pia-arachnoid. A part of the dura mater that extends as a thin connective tissue between the two cerebral hemispheres is called the falx cerebri. A thicker portion of the dura mater occupies the space between the cerebrum and the cerebellum, and it is referred as the tentorium cerebelli. A thickened dura surrounding the attachment or stalk of the pituitary gland is called diaphragma sellae (Hodges 1974, p. 421). The layer that is intimately attached or in contact with the brain is the pia mater, which is a thin layer of simple squamous epithelial cells resting on a basement membrane with supported by connective tissue intimately adhering to the brain tissue.

The space between synsacrum and first free coccygeal vertebra (synsacrococcygeal space) is the most suitable site for spinal injection (Kazemi-Darabadi et al. 2019). In this

region, the dura mater adhered to the internal wall of the spinal canal, and the subarachnoid space is large suggesting that the needle would be introduced into the subarachnoid rather than the epidural space.

12.7 Brain Ventricles

Similar to the mammals, the brain has four ventricles (two lateral, third, and fourth). The lateral ventricles are within the telencephalon and, in birds, are covered with a thin cerebral cortex layer. The third ventricle is located around the thalamus (interthalamic adhesion in sagittal section) while the fourth is present in the rhomboid fossa of the dorsal surface of the medulla oblongata. All ventricles have their own choroid plexuses that produces the CSF. The two lateral ventricles communicate with the third ventricle through the interventricular foramina. The third ventricle communicates with the fourth through the mesencephalic aqueduct (cerebral aqueduct). The fourth ventricle at the end of the rhomboid fossa communicates with the central canal of the spinal cord.

12.8 Cerebrospinal Fluid (CSF)

CSF is an ultrafiltrate of the plasma; therefore, it has lots of similarities with the plasma chemical composition. Analysis of chicken CSF indicates that it contains higher glucose and protein levels relative to mammals and slightly higher levels of chloride. Except for Na, K and Cl ions a clear CSF obtained from chickens varying in age from six weeks to two years did not reveal significant fluctuations in composition related to age differences. In addition, glucose, protein, and amino nitrogen did not fluctuate with age, the levels of both protein and glucose are far more than those found in the literature for mammals. Large molecules placed intravenously, such as insulin, could gain access to the CSF compartment in the chicken (Anderson and Hazelwood 1969).

Uehara and Ueshima (1985) mentioned that at segment 41 of the spinal cord (caudal region), the central canal expands and opens into the subarachnoid space. After that, it becomes successively narrower, and a cord-like extension unites distally with the periosteum of the pygostyle. It is demonstrated using specific dyes that CSF flows backward, like in mammals, at the caudal region of the avian body. It is well known that in mammals a bilateral jugular occlusion results in the elevation of CSF pressure. Chickens responded in a similar manner, when the jugular veins were clamped, meaning CSF pressure increased three to four times compared to the control (Anderson et al. 1972).

12.8.1 Choroid Plexus

The choroid plexus is a tuft of blood capillaries invaginated by highly proliferative ependymal cells to secrete and regulate CSF. The plexuses are present within the brain ventricles (lateral, third, and fourth). The choroid plexus and the central canal are lined with simple cuboidal or simple columnar with the presence of frequent cilia or microvilli (ependymal cells). The ependymal cells of the choroid plexuses rest on a basement membrane and a core of highly vascularized connective tissue to supply nutrients to the epithelium to secrete CSF. Junctional complexes are present between adjacent ependymal cells (zonula occludens, zonula adherens, and macula adherens) (Doolin and Birge 1969).

12.8.2 CSF Flow Pattern

Normal flow pattern of CSF is regulated by the intraventricular biologic pressure gradient created by secretion of CSF which exceeds the pressure created by its absorption in arachnoid villi (arachnoid granulations). Other sources, such as secretion by the ependyma, interstitial fluid of the brain, and ultrafiltrate of the blood, have also been reported to contribute to the formation of CSF. The routes of CSF drainage can be through the venous sinuses and lymphatic drainage. These venous and lymphatic vessels play important roles in CSF drainage and the maintenance of normal interventricular CSF pressure (Miller and Zachary 2017). The arachnoid villi (granulations) are focal extensions of the arachnoid and subarachnoid space that extend into the sagittal venous sinus of the brain. Exchange of fluid takes place between the villi and blood within the dorsal sagittal sinus. Lymphatic vessels will assist CSF drainage in the caudal part of the bird body like mammals.

12.9 Blood Brain Barrier

Blood brain barrier is present in the brain, with few exceptions within the circumventricular organs and the central canal of the spinal cord. This barrier, like elsewhere in the animal body, is composed of endothelial cells of the blood capillaries with their tight junctions, basal lamina of the blood capillary, and the astrocytic end feet processes. It is well demonstrated, by using Evans blue and horseradish peroxidase, that the blood brain barrier gradually develops during chick development. The final development culminates at one month of age (Ribatti et al. 1993). The barrier function of the spinal cord capillaries is based on the specialized system of

non-fenestrated endothelial cells and their accessory structures, including the basement membrane, pericytes, and astrocytic end feet processes (Bartanusz et al. 2011).

12.10 Cerebrum

The brain consists of the forebrain (prosencephalon – telencephalon and diencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon – metencephalon and myelencephalon) (Figure 12.6). The brain of birds is smooth and lacks gyri and sulci which is completely different than in mammals. It also lacks a clear separation between the medulla oblongata and the pons (Figures 12.7 and 12.8). The pyramidal tracts, decussation of the pyramids, trapezoid bodies, and corpus callosum are absent in avian (Batah et al. 2012; König et al. 2016, pp. 187–193). The triangular cerebral hemisphere is thin at the rostral end, while the caudal end is rounded and thick. Two small olfactory bulbs in the rostral part of the cerebrum are present and differ from those of the mammalian species (Figure 12.8). These olfactory bulbs are so closely attached to each other giving the appearance of a single structure (Gupta et al. 2019).

12.10.1 Telencephalon

Telencephalon includes the two cerebral hemispheres separated by the dorsal interhemispheric (longitudinal) fissure with a small olfactory bulb arise from each hemisphere. The lateral ventricle presents inside the dorsolateral surface of the cerebral hemisphere. It is surrounded by a thin layer of cortical substance (König et al. 2016, p.193). It has a choroid plexus which is located on the caudomedial portion of the ventricle (König et al. 2016, p.195). In the diencephalon around the thalamus is the cavity of the third ventricle which communicates with the lateral ventricles through the interventricular foramen and housed the choroid plexus. The third ventricle is connected to the fourth ventricle through the mesencephalic aqueduct.

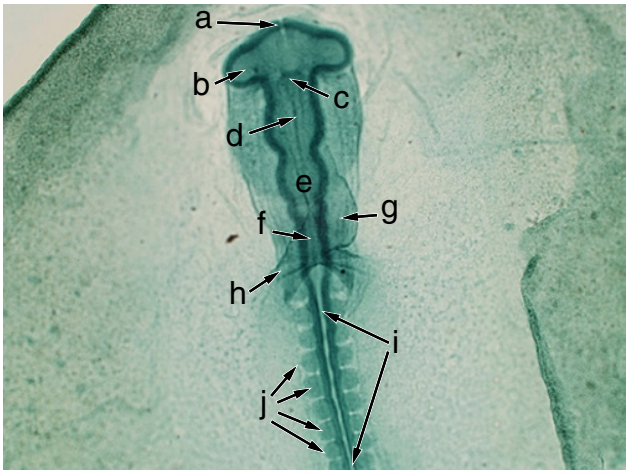

Figure 12.6 Chick embryo with the neural tube development and the body segmentations. (a) Anterior neural pore, (b) telencephalon, (c) diencephalon, (d) mesencephalon, (e, f) rhombencephalon which includes (e) metencephalon, (f) myelencephalon, (g) developing heart, (h) posterior vitelline vessel, (i) neural tube (future spinal cord), and (j) somites.

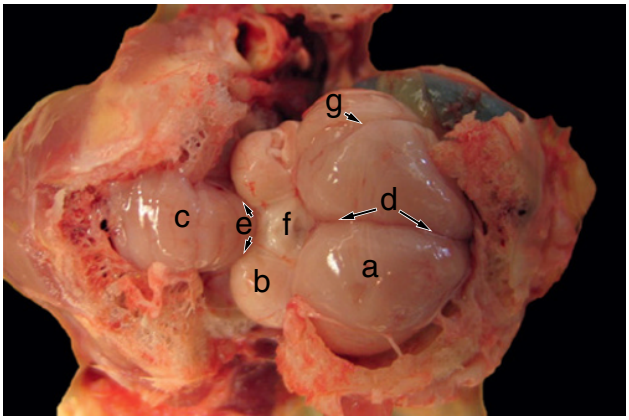

Figure 12.7 Dorsal view of the brain after removal of the calvarium and meninges showing (a) telencephalon and the label on one of the cerebral hemispheres, (b) mesencephalic tectum (optic lobe), (c) cerebellum, (d) longitudinal fissure, (e) transverse fissure, (f) attachment site for the pineal gland, and (g) groove.

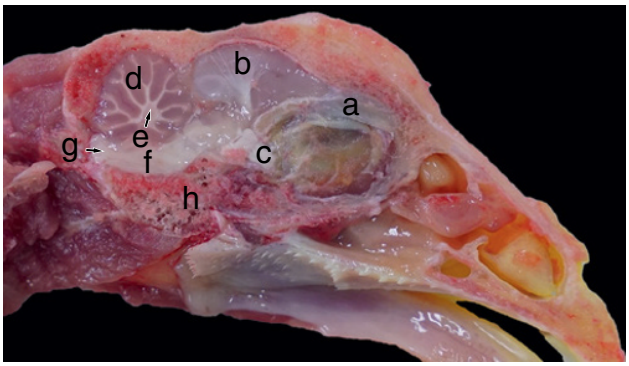

Figure 12.8 Sagittal section of adult chicken head. (a) Olfactory bulb, (b) left cerebral hemisphere, (c) optic chiasma, (d) cerebellum grey mater, (e) cerebellum white mater, (f) medulla oblongata, and (g) beginning of spinal cord.

12.10.1.1 Olfactory Bulbs

Two olfactory bulbs extend from the rostral part of the prosencephalon to occupy the olfactory fossa within the skull. Each bulb has an actual cavity representing the olfactory cavity (recess) (Hodges 1974, pp. 619–620) which communicates with the lateral ventricle and has

CSF fluid. The olfactory bulb receives olfactory axons that arise from the mucus membrane of the nasal cavity (posterior concha) in a single *filum olfactorium*. There is no ethmoid plate in avian skull but an opening for the passage of the olfactory nerve (Koch 1973, pp. 121–122).

12.10.2 Diencephalon

Diencephalon is the rostral continuation of the brain stem and consists of epithalamus, thalamus, and hypothalamus. The neurohypophysis (nervous part of the hypophysis cerebri) extends distally from the diencephalon to be close to the pars distalis (adenohypophysis) which originating from Rathke's pouch from the stomodeal cavity. The third ventricle which surrounds the thalamus extends inside the neurohypophysis in the form of a recess and into the mammillary body as infra-mammary recess (König et al. 2016, p. 193).

12.10.2.1 Thalamus

The thalamus is the largest portion of the diencephalon. It serves as a link between the brain and pituitary gland (both adenohypophysis and neurohypophysis). The dorsal part is more developed than the ventral part in chicken (Hodges 1974, p. 194). The afferent fibers arising from the body are conveyed to both cerebral hemispheres. The thalamus in mammals has two parts attached by an interthalamic adhesion. However, the latter is absent in chicken (König et al. 2016, p. 194).

12.10.2.2 Epithalamus

The epithalamus forms the roof of the third ventricle and contains its choroid plexus. The epithalamus consists of the habenular nucleus and the pineal gland embedded inside the meninges. It is located in between the cerebellum and the cerebrum which is adjacent to the dorsal surface of the thalamus.

12.10.2.3 Hypothalamus

The hypothalamus is the part of the diencephalon below the thalamus. This region has three important nuclei (supraoptic, paraventricular, and infundibular) (Hodges 1974, p. 194). These three nuclei along with the neurohypophysis form the neuro-thalamo-hypophyseal system. This system is particularly important in controlling the secretion of hormones secreted by the pituitary gland.

12.10.3 Mesencephalon

The mesencephalon, also known as the midbrain, is a region within the chicken brain that lies between the forebrain and hindbrain. It is composed of various structures and nuclei that play critical roles in sensory processing, motor control, and integration of information from different sensory modalities. One of the most important parts found in the chicken mesencephalon is the optic tectum, also known as the superior colliculus, which is responsible for visual processing.

Another significant component of mesencephalon is the mesencephalic locomotor region involved in the control of locomotion and plays a crucial role in coordinating rhythmic movements, such as walking or flying. It receives inputs from various sources, including sensory feedback and descending motor commands, to regulate and modulate motor output. Additionally, mesencephalon contains nuclei that contribute to auditory processing which receives and integrates auditory information. Motor responses may be selected or inhibited by a further evaluation of the sensory input through neural circuits involving the ascending connections of the optic tectum to the higher center in the cerebral motor cortex (Guirado and Carlos Dávila 2009).

These auditory nuclei are involved in the analysis of sound localization, intensity, and frequency discrimination. The mesencephalic aqueduct is wide and has a lateral extension deep into the mesencephalic tectum (König et al. 2016, p. 196).

12.10.3.1 Optic Tectum

The optic tectum receives visual inputs from the eyes and is involved in the detection of motion, orientation, and spatial localization of visual stimuli. Left and right optic lobes are large in chicken projecting laterally from the mesencephalon to reach postero-ventral aspect of the prosencephalon. The superior colliculi form the major portion of the optic lobe. This part is composed of many cellular elements (Figure 12.7).

12.10.4 Metencephalon (Cerebellum)

The cerebellum is located dorsal to the medulla oblongata and connected to the pons by the middle cerebellar peduncles (pontine peduncles) which are not obvious externally in chicken as they are in mammals. The rostral and caudal cerebellar peduncles connect the cerebellum to the brain stem. In general, is very similar to the mammalian cerebellum (Maulana et al. 2020). It consists of a large median lobe (body of the cerebellum) equivalent to the vermis in mammals and two cerebellar hemispheres. It is reported that the whole cerebrum to cerebellum ratio is 3.9:1 in chicken, while in humans it is three times larger ratio, because of the larger size of cerebellum in chicken (Pal et al. 2003). The cerebellum of chickens has three cellular layers: outer molecular,

Purkinje, and granular. These layers are fully developed at the incubation period of the 20th day, with a clear appearance of the Purkinje layer (Maulana et al. 2020) (Figure 12.8).

The cerebellum has a single median lobe, both corpus cerebelli with underlying flocculonodulous and the two lateral folliculi (Larsell 1948). The cerebellum may also be divided into lobes (anterior, posterior, and flocculonodular). The vermiform cerebellum is divided into three parts: anterior, middle, and posterior lobes by deep fissures with each lobe divided into numerous folia. The basal part of the anterior lobe is the lingula while the uvula can be identified on the posterior lobe (Nickel et al. 1977, p. 120). The white mater (arbor vitae) is composed of axons and other fibers. The deep nuclei of the cerebellum are embedded in white mater. They have two region-specific excitatory neuron classes and three region-invariant inhibitory neuron classes (Kebschull et al. 2020a, b) (Figure 12.9). The molecular layer has spindle-shaped, oval, or triangular cell bodies. Their axons synapse on Purkinje cells. Also, star-shaped (stellate) and basket cells are large pyramidal, or spindle-shaped neurons present within this layer. Purkinje cell layer is a very thin region between the molecular and the granular layers. Each Purkinje cell is a large, round, pyramidal, or flattened-shaped neuron. Dendrites branch as they leave the cell into primary, secondary, and tertiary forming a crown-like structure on the cell. From the base of the Purkinje cell, the axon passes into the granular layer. The granular layer is composed of compact, rounded, darkly stained nuclei. This layer extends from the Purkinje cell layer to the arbor vitae. The neuronal cell bodies present in this layer are granule cells and Golgi type II cells. Several processes project from the cell body to terminate in a tuft. These processes make interconnections with other cells within the cerebellum. Therefore, synaptic contacts among granule cell post-synaptic dendrites, and terminals of Golgi cell pre-synaptic axon terminals and dendrites surrounded by a glial sheet around the Mossy fibers of pre-synaptic terminals form the cerebellar glomerulus (Mapelli et al. 2014).

The cerebellum coordinate motor functions associated with posture and maintained body balance. It has several other functions including higher cognitive processes. It receives inputs from many organs, visual, auditory, and proprioceptive regarding body positioning. It acts according to the overall inputs that received to precisely modulate actions initiated by the cerebral motor cortex to result in fine movement. Cerebellar damage usually results in loss of coordination and hypermetria (muscular movement beyond its intended goal).

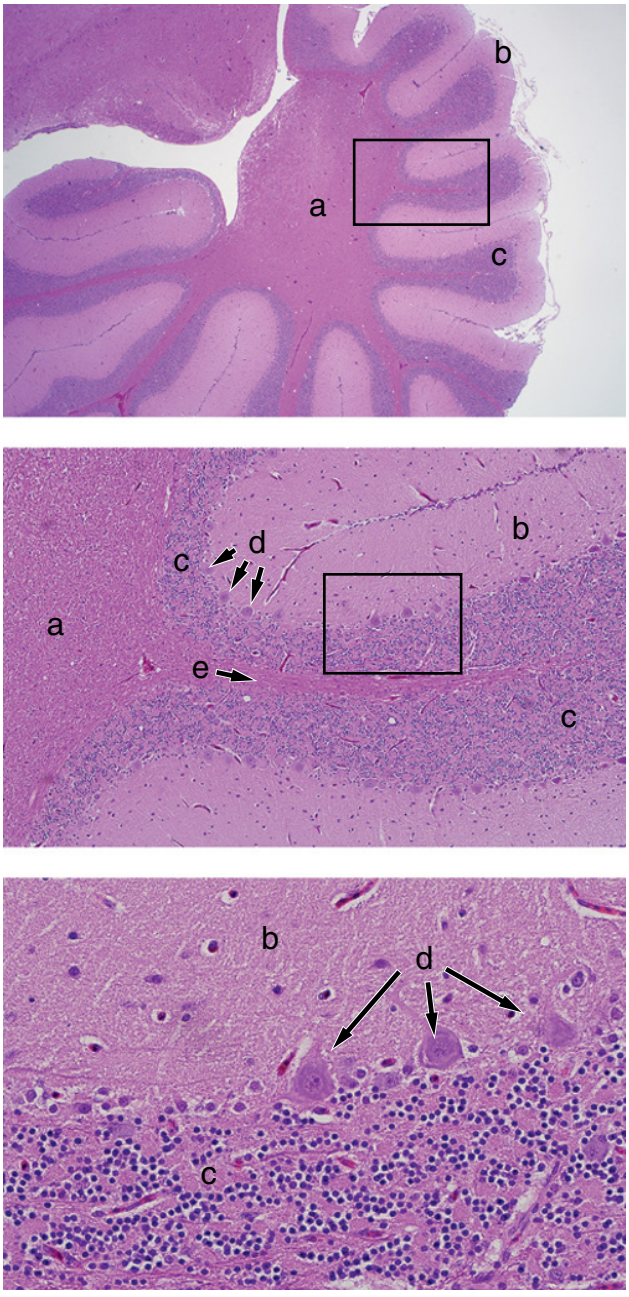

Figure 12.9 Section of the chicken cerebellum, (a) white matter, (b) molecular layer, (c) granular layer, (d) Purkinje cell layer, and (e) arbor vitae. H&E stain.

12.10.5 Myelencephalon (Medulla Oblongata)

The medulla oblongata is located caudal to the metencephalon, and it is the junction between the metencephalon and the spinal cord (Figure 12.10). On its dorsal surface, the rhomboid fossa is found where the fourth ventricle is located. The choroid plexus of the fourth ventricle projects out in the caudolateral part of the medulla

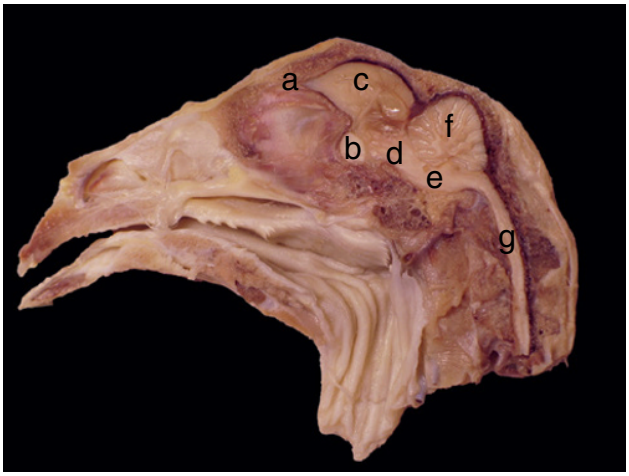

Figure 12.10 Sagittal section of a chicken head and neck (formalin fixed) showing (a) olfactory bulb, (b) optic tract, (c) telencephalon, (d) mesencephalon, (e) medulla oblongata, (f) cerebellum, and (g) spinal cord.

oblongata into the subarachnoid space through the lateral aperture that is the opening connecting the fourth ventricle with the subarachnoid space. (König et al. 2016, p. 196). The fourth ventricle caudally becomes small and ends at the obex where the central canal of the spinal cord starts. The CSF flows from the fourth ventricle to the central canal of the spinal cord. Many cranial nerves originate at the medulla oblongata.

12.11 Reticular Formation

The reticular formation refers to a network of interconnected nuclei dispersed throughout the brain stem (mesencephalon and medulla oblongata), lacking precise anatomical demarcation due to its incorporation of neurons situated across diverse brain regions. This specialized brain stem nuclei consists of neuronal cell bodies among branching dendrites present in brain stem including pons and medulla oblongata. These dendrites contact ascending and descending tracts. The reticular formation plays a significant role in breathing and cardiovascular functions (König et al. 2016, p. 191).

This region of the brain stem is related to several functions such as somatic motor control through neurons originating at the motor cortex send their axons to the reticular formation nuclei and form the reticulospinal tracts of the spinal cord. These tracts function in maintaining tone, balance, posture, and motor coordination. It is also involved in cardiovascular control through the cardiac and vasomotor centers of the medulla oblongata.

12.12 Spinal Cord

At very early stage of embryonic development, the neural tube formes from aggregations of neuronal cell bodies, neuroectodermal in origin, forming the basal and alar regions. The basal region forms the ventral horn while the alar region forms the spinal cord's dorsal horn. The spinal cord starts at the opening of the foramen magnum at the base of the occipital bone and extends inside the vertebral canal to the uropygium. The cylindrical spinal cord is divided into two identical halves by the dorsal sulcus and ventral fissure.

Spinal cord consists of grey and white matter. The most internal grey matter is arranged with the shape of a butterfly, with four "wings" called horns. The ventral horns are constituted by cell bodies of motor neurons while the dorsal horns contain the cell bodies of sensory interneurons. The grey matter of the spinal cord is enveloped by a white matter column, comprising axonal tracts that facilitate efficient communication between distinct regions of the spinal cord and CNS. These tracts enable the transmission of sensory and motor signals, facilitating upward and downward signal propagation involved in processes such as sensation and motor control.

The sites of the sensory dorsal root entrance into the dorsal horn and the motor ventral root exiting divide the white mater into dorsal, lateral, and ventral funiculi. The funiculus is a bundle of ascending or descending axons passing through the white matter of the spinal cord. Both halves of the spinal cord communicate with each other through the white and grey commissures (Jungherr et al. 1969). A spinal segment is formed by both paired dorsal and ventral nerves plus the dorsal root ganglion. The grey matter is positioned around the central canal of the spinal cord and it is peripherally surrounded by white matter. The white matter is composed of ascending tracts that occupy the dorsal funiculi, while the descending tracts occupy the ventral funiculi and both are represented in the lateral funiculi (König et al. 2016, p. 187).

The chicken's spinal cord assumes additional functions in comparison to mammals due to its elongated cervical vertebral region, resulting in a longer spinal cord (Figure 12.10). The spinal cord is bilaterally symmetrical and uniform (Hodges 1974, p. 604). It has a small central canal as a continuation of the brain ventricles where CSF flows. The spinal meninges cover and support the delicate spinal cord and are associated with the denticulate ligaments located laterally in the form of bands of connective tissue that extend from the pia mater to the dura mater. These ligaments are well developed in the cervical region. The subarachnoid space is the largest in the region between synsacrum and first coccygeal (caudal) vertebra (synsacrococcygeal space). The dura mater in this region adheres to

the internal wall of the vertebral canal increasing the subarachnoid space. This is the recommended site for epidural access/injections (Kazemi-Darabadi et al. 2019).

Afferent (sensory) axons reach the dorsal horn of the spinal cord after passing through the dorsal root ganglion, whereas the efferent (motor) neurons exit from the ventral horn. These bundles of axons of the sensory and motor are called dorsal and ventral roots, respectively. At the intervertebral foramen, the roots merge forming the spinal nerve. The exchanged fibers diverge forming the dorsal and ventral branches of the spinal nerve. Therefore, both dorsal and ventral branches are mixed while the roots are pure sensory (dorsal) and motor (ventral).

The reported number of spinal nerves are variable ranging from 39 (Koch 1973) to 41 (Ohmori et al. 1984; König et al. 2016), with 15 cervical, 7 thoracic, 14 synsacral, and 5 caudal. The first cervical nerve is reported to have only ventral roots. Starting at the third cervical vertebrae onwards the dorsal root has a dorsal root ganglion (Hodges 1974, p. 2010). There are, like mammals, two enlargements of the spinal cord where the innervation for the wing and the hind limb arise. The brachial plexus originates from the cervical or cervico-thoracic enlargement or intumescence (*intumescentia cervicalis*) of the spinal cord. It extends from about the 13th cervical vertebra to the second thoracic vertebra. The lumbosacral enlargement (*intumescentia lumbosacralis*) extends from the sixth thoracic vertebra to the sixth sacral vertebra. While Ohmori et al. (1984) stated that nerves 23–30 contribute to the formation of the lumbosacral plexus nerves that supply the hind limb in the domestic fowl. The cervical enlargement is larger than the lumbosacral in flying birds while the opposite is true in flightless birds (König et al. 2016, p. 187). Behind the lumbosacral enlargement, the spinal cord tapered down and terminate at the uropygium (Jungherr et al. 1969).

The motor axons that innervate the chick wing emerge from the spinal cord in spinal nerves 12–17. These axons grow toward the developing limb and congregate at its base to form the plexus (Dieu and Newgreen 2007). Muscles and skin of the vertebrate limb are supplied by peripheral nerves arranged in species-specific patterns. The chick brachial plexus shares many similarities with that of the human pattern despite the superficial differences of the adult arm and wing (Dieu and Newgreen 2007). The hindlimb muscles are divided into two groups which act on either the hip and knee joints or the knee, ankle, and toe joints. Muscles acting on the proximal joints are innervated by all motor neurons in the lumbar segments and by those of the dorsolateral columns in sacral segments, while the others relating to the distal joints are supplied by motor neurons of the ventromedial columns in the sacral segments. The lumbosacral plexus of the domestic fowl is usually made up of ventral rami of spinal nerves 23–30. It is divided into two parts, lumbar and sacral plexuses (Ohmori et al. 1984).

12.13 Glycogen Bodies

The glycogen body found at the lumbosacral intumescence is only present in birds. It contains substantial amounts of glycogen which is the reason behind the name. It covers the dorsal sulcus separating the spinal cord into left and right sides. It is composed of a dorsal and ventral portion by a pial layer (Dickson and Miller 1957). The body consists of a gelatinous mass of cells located at the dorsal roots of the sciatic nerves. It is found between spinal nerves 26 and 29 inclusive (Hodges 1974, p. 609) or third to sixth sacral dorsal segments separated from the spinal cord by a thin pial layer (König et al. 2016, p. 187). It is described by Ebraheim (2016) to be delicate, ovoid, circumventricular, transparent, and gelatinous glycogen body embedded in the dorsal part of the lumbosacral region of the spinal cord at the level of L2, L3, L4, S1 and S2 segments. It can easily be removed without destroying the spinal cord tissue. Its cross-sectional area is maximal at L4. Cranial to L2 and caudal to S2 the body is much smaller thus leaving a large space dorsally. The glycogen body is also reported in the cervical and upper thoracic regions; therefore, the name brachial glycogen body is proposed by Sansone and Lebeda (1976). However, Uehara and Ueshima (1982) mentioned that glycogen body is present within the entire spinal cord. Heavily concentrated glycogen at the cervical intumescence and ependymal cells were often seen throughout the spinal cord length. The glycogen body is metabolically inert and may be functionally geared to support the process of myelin formation in the avian CNS. It may also serve as a source of organic acids which might provide alternate substrates to the CNS under conditions of metabolic stress (Benzo and De Gennaro 1983). Other functions were described in pigeons like transmission of hydrostatic pressure changes during movements of the bird on the ground (Necker 1999). The existence of a blood brain barrier was demonstrated in the chick glycogen body by the exclusion of injected horse radish peroxidase and by immunocytochemical localization (Möller and Kummer 2003).

12.14 Autonomic Nervous System

The autonomic nervous system consists of sympathetic and parasympathetic parts, as described in mammals. The sympathetic portion has a short preganglionic axon

and a long postganglionic axon while the parasympathetic is the opposite. The ganglia within the sympathetic present outside the organ they supply while in the parasympathetic portion, the ganglia are remarkably close to or inside the organs walls they supply. Neurotransmitters at the preganglionic fiber synapse for both sympathetic and parasympathetic are acetyl choline while they differ at the postganglionic synapse where norepinephrine and epinephrine are for the sympathetic and acetyl choline for the parasympathetic with few exceptions (LeBouef et al. 2022).

12.14.1 Sympathetic

The preganglionic columns in avian are in the spinal cord grey matter in a dorsal and dorsolateral position relative to the central canal. It starts at the last cervical and ends at the first or second lumbar segments of the spinal cord (Getty 1975, p. 2054). However, the sympathetic trunk ganglia are aggregates of neuronal cell bodies migrated at early stage of embryonic development from the neural crest cells. The average ganglia on each side of the body are 37 (14 cervical, 7 thoracic, 13 synsacral, and 3 caudal) (Figure 12.1).

12.14.2 Parasympathetic

The origin of the parasympathetic portion of the autonomic nervous system is from the cranial and sacral regions, thus it is craniosacral outflow. Preganglionic cell bodies are present in the brain stem, for example, dorsal nucleus of the vagus and parasympathetic nucleus of the oculomotor nerve. Cranial parasympathetic fibers carried within cranial nerves III, VII, IX, and X. That is in addition to the synsacral part of the spinal cord. The first three nerves distribute in the head, while the last one (X) extends itself to supply the viscera of the cervical, thoracic, and abdominal regions in addition to the head. Several ganglia present in the head including ciliary, sphenopalatine, and orbito-nasal (Getty 1975, pp. 2054–2057).

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13

Applied Chicken Anatomy

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13.1 Examining from a Distance

Before there is any direct interaction with any chicken, it is important to examine the bird from a distance (Morishita 1999, 2021). As an observer, it is important to examine how the bird interacts with its surroundings. There should be a visual examination as well as an aural examination (Morishita 1999, 2021). An aural examination means listening to the sounds of the birds at rest and to be able to pick up abnormal breathing sounds which may indicate respiratory illnesses.

13.1.1 Daily Behavioral Deviations

It can easily be ascertained that a chicken is not clinically healthy when changes are observed in its normal behavior (Morishita 1999, 2021). For example, it is important that one observes the chicken flock daily to observe for the signs of reduced feed intake and/or water consumption. One of the first signs that a chicken may not be normal is a reduction in feed and/or water intake as a sick chicken would be reluctant to move about and may prefer remain still. If the birds are laying eggs, there will also be a cessation to the egg laying. Depending on the strain of chicken, some are expected to lay approximately one egg a day. However, birds that are physiologically stressed due to physical stress or disease will cease egg laying since egg laying is not an essential function for the individual bird's survival (Morishita 1999, 2021). The bird will stop laying eggs to focus its energies on its essential bodily functions to remain alive. If changes are noted in the normal daily behavior, it would be recommended to investigate the situation further to see if there is a plausible cause.

13.1.2 Abnormal Mentation

Birds that are not feeling well may appear lethargic and be reluctant to move. A normal chicken is always focused on its environment and external stimuli (Morishita 1999, 2021). A healthy chicken will focus their attention on external stimuli. The chicken is a prey animal so its behavioral characteristic will be observed for potential predators. If a bird does not appear to focus on a potential predator, then there should be a concern that something may be amiss.

It should be noted that chickens will often rest during the heat of the day and when an individual walks into a room of floor-raised birds, the birds may appear to be huddled together and may even appear sleeping (at rest). In fact, they may indeed be resting. To determine if the birds are just resting or if they are not feeling well, the birds should be approached slowly and then observed for their subsequent reaction (Morishita 1999, 2021). Birds at rest will eventually move as one approaches them, whereas lethargic birds will be reluctant or unable to move.

13.1.3 Abnormal Posture

Chickens that are not clinically healthy can also demonstrate deviations in their posture (Morishita 1999, 2021). It is important to observe for the signs of asymmetry. For example, birds that are resting on the ground will have their legs tucked under their bodies, so they are not easily viewed. Legs should never be outstretched in the cranial direction as this would be an abnormal position for a resting bird. On occasion, some chickens do lay on one side with their legs outstretch to stretch but this is a temporary position, and the bird should correct

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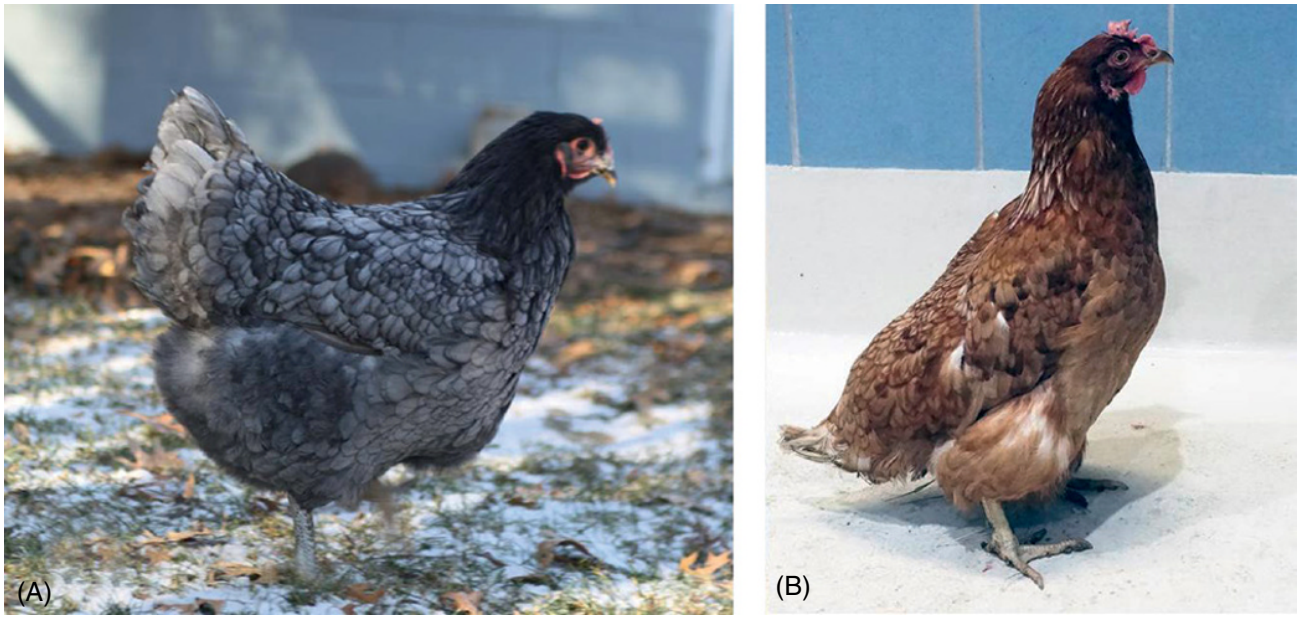

Figure 13.1 (A) The normal chicken has its thorax orientation on a horizontal axis as demonstrated in this hen. (B) A chicken with a reproductive disorder will often have its thorax oriented on a vertical axis. This is often referred to as a "penguin stance." It should be noted that this stance is normal for penguins and a breed of ducks, known as runner ducks. However, for chickens, this would be an abnormal stance.

itself to have its legs tucked under its body (Morishita 1999, 2021). Again, the leg should not extend to the cranial direction.

In the standing position, the thorax of a bird is in the horizontal axis (Morishita 1999, 2021). Some birds such as penguins and runner ducks will have their thorax upright as in an almost vertical axis (see Figure 13.1A and B). If a chicken is in a stance where the thorax is shifted to a vertical axis, then this would indicate an abnormal stance and respiratory disorders such as impacted oviducts or egg binding should be considered (Morishita 1999, 2021).

Another abnormal posture is the appearance of a tucked in head. The head of the chicken is normally held above its thorax (body). Sick chickens will tend to have their head tucked close to its thorax as in a hunched-up position (Morishita 1999, 2019a, 2019b, 2021). Oftentimes, these birds will also have fluffed-out feathers and indicate a "sick bird" appearance (Morishita 1999, 2021).

Clinically healthy chickens that are standing will also have symmetrical placement of its limb appendages when observed head-on and/or from a side view (Morishita 1999, 2021). The wings should be tucked close to its thorax and not dangling to touch the floor. As mentioned previously, a clinically healthy chicken that is sitting has its legs tucked under its body. Any chicken that is sitting and does not have its legs under its body should be considered as having mobility issues. For example, a chicken with Marek's disease will often have legs outspread in opposite directions (see Figure 13.2). In addition, chickens that have mobility issues may also "wing walk" which means using their wings to help ambulate (Morishita 1999, 2021). You will note that the wings of these birds will be dangling and often the feathers will be damaged and soiled. The wings in these cases would function as mobility aids as is equivalent to a human using crutches to ambulate.

Figure 13.2 A chicken with Marek's disease which has the classical sign of outstretched legs in opposite directions. These birds will be unable to ambulate.

13.2 Physical Examination

After examining the chicken from a distance, it is important to handle the individual to examine for any abnormal findings. Knowing the normal anatomy to access the health and welfare of the chicken is of utmost importance. The most important aspect when performing a physical examination is to be consistent and to observe and evaluate each body system (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021).

13.2.1 Feathers

One of the best methods to quickly evaluate a chicken is to observe the state of its feathers (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). A clinically healthy chicken will preen its feathers daily and the condition of the feathers will be smooth, and feathers should lay flush against its body. A chicken will obtain oil from its preen gland and will consistently preen its feathers to keep them waterproof. For birds, feathers play a major role in their thermoregulation. Sick birds will not preen themselves and their feathers will not appear smooth and contoured to their body. As discussed in feather anatomy, the actual feather vane is composed of multiple feather barbs which are composed of barbules which are joined together by hooks. A feather vane can be separated by physically separating the barbs and their associated barbules and they can then be pushed back together to form a solid feather vane again. When these feathers barbs/barbules separate naturally, it is the act of the bird preening that allows the feather to be put back together again to form a solid feather vane. Hence, if a bird is ill, it will tend not to preen, and the feathers can appear disheveled. Hence, visual appearance of the feathers is a major clue into the insight of a bird's health (Morishita 2021).

Feathers should also not have broken tips, often referred to as "moth-eaten" feathers, as this may be an indication of nutritional deficiency, such as protein deficiency (see Figure 13.3). Feathers are primarily made of keratinized protein so any deficiency in protein will affect feather growth. In fact, when there is poor nutrition, there will often be areas known as stress lines in the feathers (Morishita 1999, 2021). These would be areas in the feather vane that appear as translucent lines on the feather vane and would indicate poor feather growth during suboptimal nutritional times. These translucent areas are weakened areas and oftentimes are the locations where the feather tips would break off.

In addition, feathers should be clear of debris (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). Certain external parasites such as lice and/or mites can cause a build-up of debris on the feathers that consists of the parasites and their eggs, excreta, and/or dried blood (see Figures 13.4A, B and 13.5A, B). Lice will tend to lay whitish clumps of eggs, also known as "nits", at the base of the feather shaft (see Figure 13.4A). Mites will tend to have blackish debris on and along the feather and its shaft (see Figure 13.5A, B).

While feathers cover most of the body, it can often mask underlying issues such as muscle wasting (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). Chickens that are emaciated will have a prominent keel bone. The prominent keel bone can be felt by the handler but is often masked behind the feathers unless the chicken is physically handled (see Figure 13.3).

Figure 13.3 This is the Turken breed of chicken. The Turken is also known as the Naked-neck Chicken. While this breed normally does not have feathers on its neck, note the lack of body feathers, presence of moth-eaten feathers, and the prominent keel bone which indicates the poor nutritional state of the bird which was subsequently euthanized for humane reasons.

Figures 13.4 (A, B) These photos are examples of feather lice (insects). (A) Showing the active lice (chicken body lice) that are visible to the naked eye. (B) Showing the clusters of lice eggs that are usually glued to the base of the feather shaft, especially prominent on the second feather from the right.

Figure 13.5 (A, B) These photos are examples of the most common feather mite, the northern fowl mite. (A) Mites are much smaller than lice, but to the careful eye can be observed traversing the feathers. (B) Displaying the debris along the feather shaft which includes remnants of mites, mite eggs, their excreta, and/or dried blood.

13.2.2 Head Region

Observation of the head region is important and can help to provide an assessment of the bird's overall health. As one observes the head region, it is first important to look at the chicken's unfeathered portions of its head. These areas include skin ornaments, the comb, and the wattles. There are many different breeds of chickens and there are a variety of comb types. In fact, chicken breeds are often distinguished by comb types (Morishita 1999, 2021). The various comb types can include such varieties as single comb, pea, rose, V-shaped, and buttercup. Some combs are very close

to the body, whereas others extend outward. For any of the comb types, it should be uniformly red in color. While the shade of red may vary with age, the color should be uniform (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). Sometimes there can be areas of discoloration. For example, chickens that have respiratory diseases can have combs that are darker in color. It is often referred to as a bluish discoloration and represents a lack of oxygenation due to respiratory diseases that would impact oxygen exchange. Another potential color discoloration is a white and often flaky comb which can be associated with a fungal disease known as flavus (ringworm). If a discoloration is noted in the chicken comb, it is important to seek a veterinarian's advice.

Other issues that may be observed on the comb of birds is scabbing (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). The scab represents areas where there were breaks in the skin and could have been open wounds that have healed. On occasion, some individual birds can be aggressive to one another, especially under crowded conditions. However, there should be a few random areas of scabbing usually associated with the distal comb regions. If pen fighting is an issue, there should be more scabbing or injury on the combs of lower-ranked birds that are often picked on by higher-ranked birds in the flock. This infighting is due to the pecking order, a normal behavior in chickens. Chickens need to establish a rank order whenever flock dynamics change such as the addition of new birds to the flock. It should be noted that birds that have single combs may have more potential to comb injury as their combs extend farther from the head when compared to comb types that do not extend as much from the head (Morishita 1999, 2021).

The other concern with the presence of scabbing is that it could also represent clinical signs of a disease known as fowl (avian) pox which is caused by the virus (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). This virus enters the bird's body though it breaks in the epithelial (skin) surface or mucosa of the oral cavity. When the virus enters these breaks in the skin or mucosal surface, it will cause a localized reaction and a scab will develop at the site of entry. A scab would appear for a skin lesion break, or a white plaque would be noted for a break in the mucosal surface of the oropharyngeal cavity. It is important to observe the region of scabbing. Pox scabs will occur at any location of the unfeathered bird's body so the tip and base of the comb may be affected as well as along the margins of the eyes. Hence, it is important to observe the number of birds in the flock affected and the distribution of the scabbing as this will assist in determining if there is a disease outbreak or just scabbing from occasional pen mate aggression.

Finally, another observation that can occur on the comb is necrosis of the comb tips (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). The necrosis is especially noted in single comb chicken breeds. These sites of necrosis would occur only on the tip of affected chickens. Some chickens will have darkened tips with a sharp demarcation between healthy red comb tissue and the dark, often shriveled areas of the comb tip. These are typical signs of frostbite. These birds would have a history of being kept outdoors in inclement weather or during cold freezes. Hence, it is always important that chickens be provided with warm areas to roost when kept outdoors. If you find necrosis of the tips of the comb and do not reside in areas where the temperature drops to freezing levels, then there is one other situation where necrotic tips can be observed. Comb tip necrosis has been associated with the presence of the mycotoxin ergot. Hence, if there is no history of inclement weather and there are multiple birds affected, a possible ergot contaminant should be investigated. While this contamination is rare, it should be considered if necrotic comb tips are observed in chickens.

13.2.3 Wattles

The wattles can sometimes be swollen and can represent a local wound infection as an abscess. Roosters will have larger wattles present when compared to females. As with any fleshy skin appendage, it can be lacerated or injured during aggressive fighting, but in general there are few lesions and/or disease scenarios associated with the wattle of chickens. The only disease that has been noted in the wattles is avian (fowl) cholera caused by the bacterium *Pasteurella multocida*. This is usually observed in older male roosters and represents a chronic form of avian cholera (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021).

13.2.4 Eyes

The eyes of chickens should be clear of mucus and excessive fluid. Birds that are ill may have excess fluid in their eyes that sometimes can appear to contain tiny bubbles. If the chicken does not presently have excess fluid (tears) in their eyes, look for evidence of dried mucus, soil or dirt that may have once adhered to the mucus around the eye and/or nasal region.

The area surrounding the eye should also be evaluated for swelling. There are several respiratory diseases that would cause generalized swelling around the eye, including Newcastle disease, avian influenza, and infectious coryza (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). In addition,

some respiratory diseases may just cause swelling of the antorbital (infraorbital) sinus. This sinus is located cranial and ventral to the eye and can swell up, sometimes both eyes are affected and other times, only one side of the eye will be affected. Since its only drainage canal is located dorsal to the sinus, drainage of the fluid accumulating in this sinus cannot happen naturally and the fluid accumulates. Sometimes it can become caseated and a hardened plug can be palpated in this region. It must be removed via surgical intervention if desired to improve cosmetic appearances for the affected bird (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021).

After observing the areas surrounding the eye, the eye proper can then be observed. The pupil margins should be circular and the edges smooth and uniform. The iris is a bright yellowish orange color in most chickens. Sometimes, the iris may be gray in color. This gray color may indicate that the bird may have had Marek's disease and is often called "gray eye" syndrome in those birds that have survived the infection (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). Since Marek's disease spreads rapidly in the flock due to contaminated feather dander, multiple birds may be observed with this syndrome.

Eye injury can also result in eye discoloration and an irregular pupil margin, but the key is to evaluate your flock of birds as an individual bird affected will usually indicate more of an individual bird problem as opposed to a flock-wide disease situation (Morishita 1999, 2021).

13.2.5 External Auditory Canal (Ears)

The external auditory canal (ears) of a chicken can be found easily by following the eye and moving in a straight line toward the nape of the chicken. The ear is usually covered by feathers and in some breeds of chickens, such as Araucana, with tufts of feathers (Morishita 1999, 2021).

There are usually no clinical issues associated with the ears of the chicken. In chickens kept under poor management conditions, there can be the possibility of having maggots present within the auditory canal (Morishita 1999, 2021).

Most chicken breeds will also have a fleshy non-feathered area ventral to the external auditory canal that is often referred to as the earlobe. It is often either red or white in color. It has often been noted that for most chicken breeds, those that had white earlobes lay white eggs and those with red earlobes lay brown eggs rather than based on the color of their feathers (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). For example, an all-white feathered bird with red earlobes will lay brown eggs while an all-white bird with white earlobes will lay white eggs.

13.2.6 Thorax

While the thorax of the bird is primarily covered in feathers, it is important to palpate the breast region to assess nutritional status (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). Also, since the skin is transparent, the keel bone can often be observed under the skin if the bird is not excessively fat. In these cases, the keel bone can also be observed for any deviations in its curvature which would indicate potential issues with calcium deficiency, that is, rickets or osteomalacia.

Chickens that spend excessive time on the floor can also develop an inflamed breast bursa, often referred to as a breast blister that can become irritated and often infected, resulting in a localized infection and subsequent cellulitis (Morishita 1999).

The crop region can sometimes be filled with undigestible material and become enlarged. This can be visualized as an enlarged area in the region where the neck enters the thoracic inlet. On occasion, the crop can become greatly enlarged due to the present of excessive foul-smelling ingesta and fluid, and the crop can be seen hanging from the neck of the bird. This condition is referred to as pendulous crop (Morishita 1999, 2021).

In the caudal ventral thoracic region, the open ends of the pubic bones should be identified. Sometimes, it is necessary to determine if a chicken has laid eggs before (hen) or has never laid eggs (pullet). To distinguish if a bird has laid eggs previously can be difficult to assess via visual inspection alone. The bird will need to be physically palpated. A chicken that has laid eggs before will have a three-finger or more width of space that separates the two open ends of the pubic bones. A pullet, which has not laid eggs previously, will usually have a one- to two-finger width separating the open pubic bones. This is a quick way to determine if the chicken has laid eggs previously (Morishita 1999, 2021).

13.2.7 Cloaca

Finally, the cloaca should be observed. It should be clean and free of fecal matter (see Figure 13.6A). Birds that have severe diarrhea often have fecal matter adhering around the feathers of the vent region (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2019b, 2021). This condition is often referred to as pasty vents (see Figure 13.6B). Pasty vents are very prominent in young chicks with the dark fecal staining shown in contrast to their yellow down. In older birds, pasty vents could also be observed more easily if they are white-feathered.

The only other debris that is associated with the cloaca is a condition known as vent gleet (Morishita 1999, 2021). This is a whitish debris from the urinary system and

Figure 13.6 (A, B) The cloaca should be clean and free of any fecal material (A) The cloaca is the common site where the digestive, reproductive and urinary tracts meet. (B) Demonstrating a chick with a pasty vent in which fecal material is adhered to the cloacal region

represents leakage of urates from the bird. Sometimes damage to the internal lining of the urodeum from egg laying can result in this condition in laying hens.

Other abnormalities that can be observed in the cloaca region include a prolapsed cloaca. This can occur in young chicks with excessive diarrhea. These birds will have a prolapse of the cloaca (Morishita 1999, 2021). Unfortunately, for birds that are housed with other birds, the prolapsed cloaca with its fleshy red tissue can be attractive to the pen mates which can begin to peck the prolapse tissue. Sometimes, it can get very severe that intestinal segments can be noted, and the chick should be euthanized.

This cloacal prolapse can also occur in older hens that may have calcium deficiency because of heavy egg production levels. If the hens are not supplied with enough calcium in their diet, increases in the incidence of cloacal prolapses can occur (Morishita 1999, 2021). In these cases, during the egg laying process, there is an eversion of the cloaca. Hens with calcium deficiency will have a prolonged return of the tissue and/or there will be a tissue prolapse. Pen mates see this everted tissue, become attracted to the tissue, and peck away at the tissue-causing tissue necrosis and often time prolapsed sections of the intestines. These birds, like the cases seen in chicks, should be euthanized as prognosis is poor and the tissue is often necrotic or missing. A lack of sufficient nest areas can also exacerbate this problem in the flock.

13.2.8 Legs

The legs of chickens are covered with keratinized scales (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). The scales should be closely adhered to the leg (Figure 13.7A). Pale leg color can indicate a vitamin A deficiency. If vitamin A deficiency is suspected, other clinical signs should be observed to evaluate if a vitamin deficiency is indeed occurring as well as collecting a diet history.

The parasite *Knemidocoptes mutans*, known as the scaly leg mite, can cause scales to be upturned and elevated (see Figure 13.7B). The areas underneath these scales will have beige colored debris (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). In very severe cases, affected digits can necrotize and fall off.

Some chickens do have feet that are feathered, and feathers can be seen between the scales. Some owners pull the feathers out, but caution must be taken to ensure that feather follicle damage does not occur as feather cysts can subsequently occur and can become infected (Morishita 2021). It is not recommended to remove feathers if at all possible. Feather-footed birds should be kept in environments that are dry and not muddy where mud can cake up on their feathered feet. Removal of feathers is not recommended, and owners should consider other breeds if ideal management conditions cannot be maintained.

Frostbite can also occur on the feet of chickens that are kept in cold and/or wet conditions but can be easily prevented with proper housing to protect birds from extreme cold and wet conditions (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021).

Since chickens spend the majority of their time on the ground, the condition of their flooring plays an impact on foot pad health (Morishita 1999; Greenacre 2021; Lossie and McDermott 2020; Morishita 2019a, 2021). A common lesion found in chickens raised on the flooring, especially

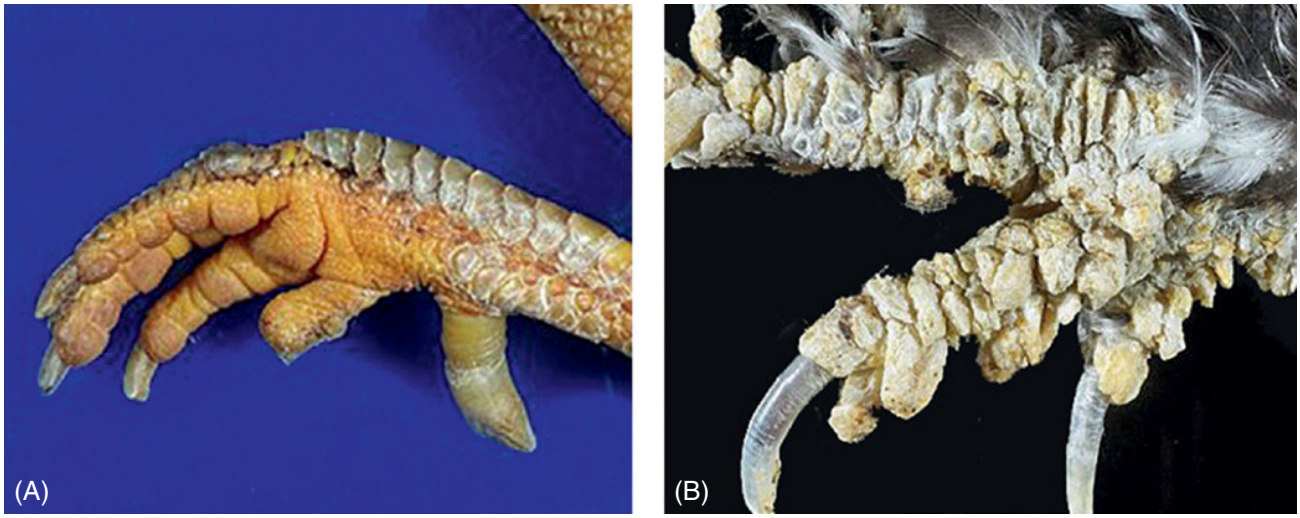

Figure 13.7 (A) Showing a clinically healthy chicken (rooster) leg. Note the scales are closely adhered to the leg. (B) Illustrating some scales are thick and separated due to infestation by the scaly leg mite.

Figure 13.8 This photo depicts the signs of bumblefoot (pododermatitis) in which the foot pads have darkened and swollen areas. These lesions will cause ambulatory issues for the bird.

damp litter, is the disease condition known as bumblefoot (see Figure 13.8). Bumblefoot, known as pododermatitis, occurs when there are micro abrasions to the ventral surface of the footpad which can be due to exposure to damp flooring, often with high ammonia level content. This condition often occurs when there are poor management conditions, and the litter is not maintained, and caking occurs. Micro abrasions lead to invasion of the wound with bacteria such as *Staphylococcus aureus*, from the skin flora, or *Escherichia coli*, from the feces, which eventually cause a localized abscess. The abscess on the footpad causes locomotor issues and a change of foot pressure for the remaining foot which can eventually develop bumblefoot. Birds with bumblefoot will have blackened, swollen foot pads. These lesions need to be treated locally and systemically.

13.3 Specialized Clinical Procedures

13.3.1 Identification Placement

On occasion, individual birds need to be identified for educational and/or research purposes. In a research setting, individual birds can be identified with the use of wing tags or leg bands (Morishita 1999).

A wing tag is inserted into the patagium of the wing. This area is an area of thin skin that has a minimal number of blood vessels. The metal tag is inserted in the patagium and clamped on. This will remain on for the life of the bird. The wing tag should be placed midway of the patagium when the wing is outstretched to avoid damaging developing wing muscles and away from feather tracts (pterylae).

Another method of identification is a metal ring that can be placed on the leg of the bird. Caution must be taken when using a static metal ring as the ring should be of the proper diameter and should be adapted as the bird grows. Static metal bands should only be used in adult, fully grown chickens. An often-popular leg band is the expandible plastic leg band which allows for expansion as the bird grows. These leg bands are usually administered to birds after their feathers have fully developed. If identification of young chicks is needed, then the metal wing bands are preferred for young chicks.

Since both these identification methods require the bird to be physically handled to read the identification, females are banded on the left legs while males are banded on the right leg for easy identification by sex of the birds before the secondary sex characteristics develop.

While microchipping has been proposed for other animals, the subcutaneous placement of the microchip

behind the neck has been met with mixed results due to the loose subcutaneous skin of the chicken and the potential for the microchip to migrate (Morishita 2021).

Other methods of identification include the color of tail and/or flight feathers via direct painting or using imping as seen in raptor rehabilitation programs. This will aid quick identification via distant visualization to minimize individual handling. However, this may impact the behavior of birds or may have deleterious effects. For example, a red painted feather may lead to pen mates singling out a chicken because of the color attraction and potential cannibalism (Morishita 1999).

13.3.2 Culture Techniques: Choana, Oropharynx, and Trachea

A choana culture is used to evaluate upper respiratory infections (Morishita 1999, 2021). To perform a choana culture, the mouth of the chicken is opened and the choana is identified and swabbed with a sterile swab (see Figure 13.9A, B).

In live birds, the oropharyngeal cavity can also be swabbed, and the swabs submitted for polymerase chain reaction tests for various poultry diseases. While a tracheal culture can be obtained in live birds by passing a swab though the upper portion of the trachea, this is not routinely performed in live birds due to availability of appropriately sized sterile swabs. In addition to the specialized reduced size needed to swab the trachea, there is also the potential for contamination in a moving, live bird. A tracheal swab, however, can be performed frequently on birds that are necropsied (Morishita 1999, 2021).

13.3.3 Blood Collection

There are certain occasions that require the collection of whole blood samples. Whole blood is also collected when plasma or serum samples are needed. There are a couple locations on the chicken that can be used to collect blood samples. It should be noted that the blood vessels of chickens are unlike those found in mammals as they are rather thin-walled in avian species (Morishita 2019b). As a result, hematoma formation is very common in birds. Bruising after bleeding is often noted as blood escapes the vessels as the needle is removed in the process. Hence, it is important to compress the vessel as the needle is withdrawn to minimize the bruising and hematoma formation.

The main sites of blood collection are the brachial vein in the wing, the jugular vein in the neck, and the medial metatarsal vein of the leg (Morishita 1999, 2019b). Each site will be discussed, and its use varies with the age of the bird, personnel available to assist, and the amount of blood samples needed.

Before collecting blood, it should be noted that there is a maximum amount of blood that can be collected from a clinically healthy avian species without causing the bird additional harm. It is estimated that 10% of a bird's weight in grams is equal to its total blood volume (mL). The maximum amount of blood that can be collected from a bird cannot be more than 10% of its total blood volume (mL). Hence, in a clinically healthy bird, the maximum amount of blood that can be collected without causing detrimental health effects is 1% of its body weight (g) (Morishita 1999, 2019b). Hence, one-day-old chick that weighs 54 g cannot have more than 0.54 mL of blood collected. It should be noted

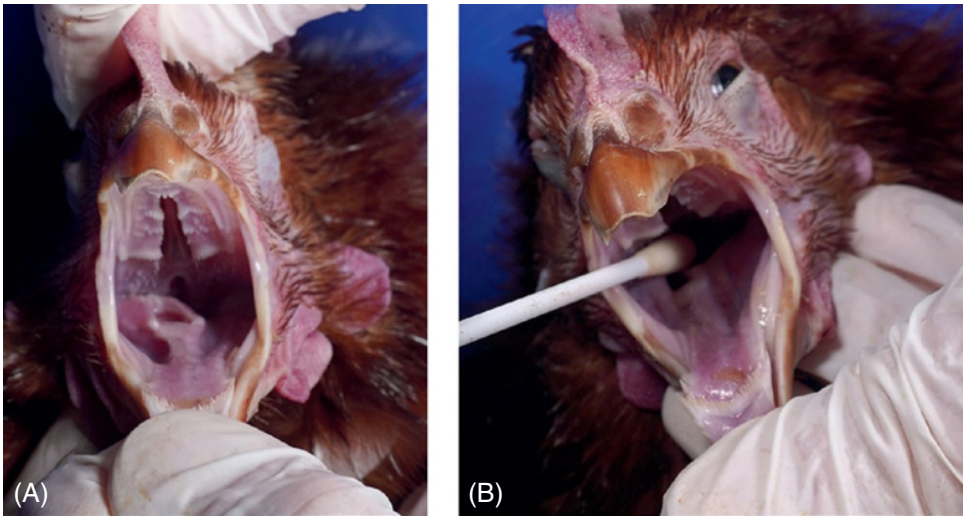

Figure 13.9 (A) Depicting an open mouth of the chicken which has a prominent cleft on the roof of the palate. This is the choana and is often the site to culture if a choana sample is warranted. The opening to the trachea (glottis) is behind the tongue. (B) Demonstrating a swab sample of the choana.

that birds that are ill and not clinically normal should have a reduced amount of blood harvested. Knowing this information and the maximum volume of blood that can be removed is important when considering the types of analyses needed.

Another factor that plays a role in the amount of total blood sample needed is the amount of sample that is ultimately harvested. For example, if serum or plasma is desired, a good rule of thumb is that 50% of the whole blood collected will yield serum (or plasma) available for testing once the blood is clotted (Morishita 2019b). Hence, these calculations should be considered to determine the appropriate sample amount needed.

A common site for blood collection has been the wing vein (see Figure 13.10A). This location has been used for routine collection and up to 3 mL can be taken successfully from this site. This site has been used for adult chickens. However, the brachial veins are too small for chicks, juvenile, and bantam-sized birds (Morishita 2019b).

In the case of one-day-old chicks, chicks, juvenile, or small stature birds, the right jugular vein is the preferred collection site (see Figure 13.10B). The right jugular vein is much larger than the left jugular vein. In addition, the jugular vein is recommended if larger volumes are needed as the vessel is larger and blood will be collected much easier than through the brachial vein (Morishita 2019b).

Finally, there is the medial metatarsal vein of the leg that can be also accessed for blood collection. However, this collection site may not yield the larger volumes that may be needed and may be used if a small amount of blood is needed. It is often used if access to the other more accessible sites is unavailable (Morishita 2019b).

A toenail clip can be used for a drop of blood but is not recommend for hematologic diagnostic testing and has been used in the past for rapid agglutination tests only (Morishita 2019b).

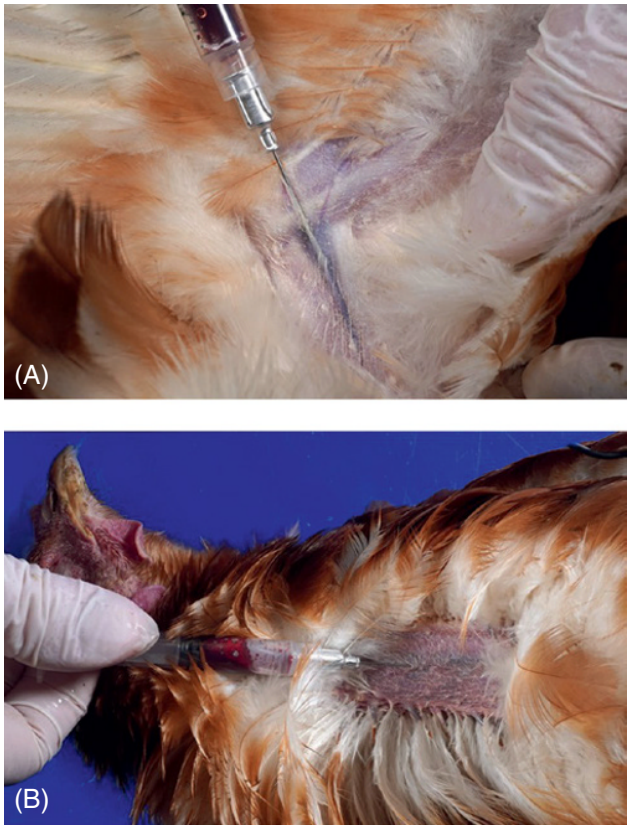

Figure 13.10 (A) Depicting blood collection from the brachial (wing) vein. (B) Depicting blood collection from the right jugular.

Hence, knowing the normal anatomy of the chicken is important and has clinical application used to access general health of the bird as well as for the collection of diagnostic samples.

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14

Chicken Necropsy

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14.1 Introduction

Keeping poultry flocks healthy and disease-free is essential for maintaining successful commercial practices, human and animal health, food supply, national and international economies, and animal welfare. For the poultry or the avian veterinarian, this requires a deep knowledge of avian anatomy and physiology, dissecting skills, biosafety practices, laboratory diagnostic skills, and an in-depth understanding of the clinical signs and macroscopic changes associated with the pathophysiology and pathogenesis—in cases of infectious agents—of avian diseases.

Avian necropsy is one of the most simple and powerful tools veterinarians can count on to rapidly identify potential health threats to the poultry industry, backyard flocks, and companion chicken. Necropsy, from the Greek words necro—"death, corpse" and opsis—"a sight, to watch," is defined as the postmortem examination of avian cadavers with the purpose of identifying the pathological and histopathological lesions that caused the death of an animal. Furthermore, by systematic sampling and investigation, avian necropsy can provide the opportunity to determine what caused the problem and/or death of these animals. Avian necropsy also allows veterinarians to perform a wide range of ancillary diagnostic tests aimed to identify the etiology and the final diagnosis of the disease/s. Ultimately, this should contribute to actions aimed to reducing morbidity and mortality, preventing disease transmission, enhancing disease surveillance, implementing potential therapeutic interventions, establishing immunoprophylaxis, addressing legal aspects, advancing scientific knowledge, and establishing proper quarantine measures.

When performing a necropsy, veterinarians rely on multiple approaches to identify the causes of morbidity and mortality in poultry farms, backyard chickens, and chickens kept as pets. Revision of management practices, production indexes, assessing facilities, identifying biosecurity deficiencies in the farm or flock, early recognition of clinical signs, and epidemiological assessment of any problem are necessary to determine morbidity and mortality. A complete and detailed *anamnesis* (case history) and review of the facility practices (feeding, immunoprophylaxis, medications, overall management of flocks, etc.) provide additional information that will help to identify potential problems.

As Louis Pasteur used to say, "*Chance favors the prepared mind*". It is not just only important for the poultry veterinarian to be aware of the most common diseases affecting poultry, but, possibly equally or even more important is to be able to recognize those that are foreign to their province, state, country, or region. The early recognition of clinical signs and postmortem changes caused by exotic avian diseases could have an enormous impact on poultry production economy and, in some cases, even on human health. Fluid communication with local, state, provincial, and national animal health authorities will also increase the likelihood of early detection of reportable and/or exotic diseases and allow quick intervention and implementation of outbreak control measures.

14.2 Euthanasia and Carcass Disposal

Although several medical conditions affecting the avian flocks are accompanied by variable degrees of mortality, occasionally avian veterinarians may need to act rapidly and perform necropsies on sick chickens that are euthanized for the purpose of observing early macroscopic lesions and collect tissue samples for histopathology, toxicology, molecular, and serological diagnosis, among

Anatomy and Histology of the Domestic Chicken, First Edition. Edited by Wael Khamas and Josep Rutllant.

other ancillary diagnostic modalities and tests. Thus, euthanasia of an appropriate number of birds with evidence of clinical and subclinical disease may be necessary. Euthanasia, the pious death of an animal, should always follow animal welfare regulations. Several methods of euthanasia have been recommended for chickens by the American Veterinary Medical Association Panel on Euthanasia (AVMA 2020). The reader is directed to this reputable source of information for specific details on how to perform these procedures. Adequate handling and restraint techniques to minimize stress for the animals are important considerations. Appropriate methods of euthanasia in chickens include gas inhalation, manually applied blunt force trauma, cervical dislocation, decapitation, electrocution, gunshot, captive bolt, and overdose of barbiturates. Death in these animals should be adequately verified, and ideally, a second method of euthanasia is recommended, such as cervical dislocation, after an individual administration of gas or barbiturates. For euthanasia of a single or a few specimens, including pet chickens, the author recommends a combination of gas anesthesia (Isofluorane or Sevofluorane) overdose (5%), followed by intravenous or intracardiac injection of a pentobarbital sodium/penhytoin sodium solution. This makes euthanasia less struggling, allows for additional pre-mortem sample collection (e.g. blood can be easily collected from an anesthetized bird), and reduces the volume of barbiturates used, which can easily precipitate and modify tissue structures, for example, if given intracardially.

14.3 Chicken Disposal

Appropriate disposal of chicken remains will result in the prevention of disease transmission to other living organisms, either in the case of animals that may have died of infectious causes, but also because of drug administration, toxins, and anesthetic/euthanizing agents (AVMA 2020). The final disposition of cadavers may require burial, incineration, composting, or rendering. Regulations on how to dispose of dead chickens may vary depending on state, province, and national laws and the appropriate regulatory organization or agencies should be consulted.

14.4 Facilities and Materials Needed

Ideally, all avian necropsies should be carried out in the pathology laboratories with strict implementation of biosecurity measures. Adequate illumination is of great value when performing necropsies. The presence of LED surgical lamps placed 50–70 cm above the pathologist offers the advantage of providing natural color rendition and minimizing reflection and shadows that can obscure tissue and lesion interpretation. The list of materials required for necropsy, sample, and tissue collection includes the following: plastic flasks with tight caps, plastic cutting boards, necropsy instruments such as Mayo, Metzenbaum and Iris scissors, scalpels, bone cutters (Rongeurs), Adson and dissection forceps, Halsted hemostats (straight and curve), intestinal clamps, sterile swabs and gauze, pathology cassettes, glass slides for smears, zip-lock bags, cover slides, syringes and needles for aspiring fluids, and a Bunsen burner. Unerasable markers and graphite pencils are useful to label specimens, cassettes, and flasks. Ideally, necropsy notes describing the main findings for each organ or lesion (location, distribution, size or extension, color, shape, texture, and consistency) should be taken consistently, with legible handwriting, and being self-explanatory. Waiting until the completion of the necropsy to write down notes is not recommended. Hand-free activated voice recorders and video recording devices can be used to further document the necropsy procedure and findings, allowing additional review of findings. To conduct photographic documentation of diagnostic findings, a small photographic chamber, with a solid, usually black background and a mounted, good quality, digital camera with flash is necessary. In addition, having a computer to upload all the photographic and other information available to electronic databases is the most convenient and practical way for archiving, reviewing, and sharing this information.

14.4.1 Personal Protective Equipment

Personal protective equipment, including disposable gowns, booties, N95 masks, hats, and gloves, is mandatory when performing necropsies, since many avian infectious diseases have zoonotic potential. Additionally, this prevents the spread of infectious agents to other animals. When an infectious agent is suspected or confirmed, and when birds died or were euthanized due to previously confirmed or suspected infectious cases, the necropsy should ideally be conducted within a laminar flow biosafety hood. This significantly reduces the risk of disease transmission, particularly in the case of zoonotic infectious agents such as aspergillosis, chlamydiosis, salmonellosis, mycobacteriosis, and certain viral diseases.

14.4.2 Histopathological Examination

The histopathological examination of organs and tissues collected during the necropsy requires adequate preservation of representative tissue sections, usually

including areas that appear to be macroscopically both normal and pathological. In most instances, neutral buffered formaldehyde 10% solution is used for organ and tissue preservation. Davidson's (sometimes called Hartmann's) fixative solution, a mixture of buffered formaldehyde 1% and glutaraldehyde 1.25%, and other special solutions may be required for ophthalmopathology. Alcohol, acetone, and heath fixation can be used for smears. Bones can be fixed using 10% formaldehyde although other specialized fixatives such as acetic acid–zinc–formalin are sometimes recommended.

Using old, blunt, rusty, or poorly maintained instruments for necropsy is not a good practice. Collection of tissue samples and accessing the interior of parenchymatous organs require the use of sharp instruments to avoid artifacts and damage to the tissues. Therefore, the use of good quality instruments, although not necessarily overly expensive, is recommended.

14.4.3 Collection of Microbiological Samples for Culture and Molecular Diagnostics

In poultry production, diseases with clinical presentations are accompanied by variable morbidity and mortality. One of the most common causes of disease in chickens is avian pathogens (bacterial, fungal, and viral). Therefore, the use of classical microbiological and molecular diagnostic methods is an essential component of any necropsy in order to reach an etiological diagnosis. For this purpose, proper collection of samples from tissue lesions, exudates, transudates, blood, and other fluids (nasal, tracheal, celomic, cerebrospinal, among others) is necessary. This will allow proper culture, isolation, and identification of most species of bacteria, fungi, and viruses in the microbiology laboratory. Samples for microbiology, either for culture or molecular diagnosis, must be collected from the nares, nasal cavity, conjunctival sac, mouth, upper trachea, and cloaca, regardless of the presence or absence of secretions or exudates (Figure 14.1). Preferably these samples should be collected before the necropsy advances to avoid cross-contamination.

For parenchymatous organs located in the celomic cavity of chickens, external surface contamination is a common problem while accessing this cavity. In these cases, dry heating of a spatula, or scalpel blade, would heat sterilize the outside of the target organ and kill contaminants located on its surface. Subsequently, a sterile scalpel can be used to incise the organ and swab the organ interior. Sterile needles and syringes should be used to collect blood, exudates, transudates, or any other fluid present in cavitary organs (e.g. heart, gall bladder). An adequate supply of different size sterile swabs and transport (Amies, Stuart, with or without charcoal, and anerobic) is necessary for bacteria and fungi. Samples collected for virus isolation require specific virus transport media and immediate refrigeration and/or freezing. An early examination and preliminary identification of certain infectious agents can be done by collecting additional swabs and preparing smears for in-house staining with Gram and Ziehl-Neelsen stains (bacteria) and Methylene blue or Indian Ink (fungi).

Figure 14.1 Collection of samples for microbiology, either for culture or molecular diagnosis, from the oral cavity (left) and conjunctival sac (right) regardless of the presence or absence of secretions or exudates.

14.4.4 Molecular Diagnosis of Microorganisms

For the molecular diagnosis of microorganisms, based on nucleic acid (DNA/RNA) identification, it is important to understand that heat sterilization or simple chemical sterilization does not eliminate nucleic acids on instruments. Sterile swabs are assumed to be nucleic acid-free and can be used to collect samples for molecular diagnosis. However, during necropsy, contamination of instruments with nucleic acids from tissues handled during previous necropsies is common, even if the material was cleaned and heat sterilized. This problem is often overlooked, and samples inappropriately collected can yield false positive results when using molecular methods. For example, using the same forceps and scissors to access the celomic and respiratory cavities will lead to contamination with nucleic acids on the skin surface or different organs on both of these cavities. Moreover, if a particular microorganism needs to be identified to understand its distribution in different tissues and organs, it will be necessary to use different nucleic acid-free instruments for each one of these tissues and organs. Otherwise, nucleic acids carryover will inevitably occur.

For organs or tissues manipulation, sampling, and subsequent nucleic acids amplification, it is recommended to have several kits of sterile and nucleic acid-free instruments (2 scissors, 2–3 forceps, 4–5 swabs, 4–5 Eppendorf or cryotubes in individual poaches) ready for this type of sample collection. First, all instruments should be cleaned and sterilized as usual after any use or manipulation of any tissue or organ during necropsy. Second, all necropsy instrumentals intended for collecting samples for molecular diagnosis can be soaked either in sodium hypochlorite solution for 20–30 minutes or in formaldehyde 10% solution overnight followed by a thorough rinsing with nucleic acid free water. In both cases, the bleach and/or formaldehyde will degrade nucleic acids into very small fragments, thus preventing subsequent carryover Nucleic Acid amplification by polymerases in a PCR and, ultimately, cross-contamination. Once these instruments are clean and nucleic acid-free, they can be included in disposable pouches and sterilized for further use. Representative sections of each organ can be saved in the cryo- and/or Eppendorf tubes using designated forceps and scissors to collect them.

14.4.5 Toxicological Investigation

For toxicological investigation, sections of major organs and gastrointestinal content can be placed in double zip-lock bags and/or 15–50 mL tubes. By subdividing and saving samples in multiple sections, we are preventing the whole organ or tissue from being exposed to multiple thawing and freezing in case further investigations are needed. For Scanning Electronic Microscopy, Transmission Electronic Microscopy, Immunofluorescence, and Immunohistochemistry, the referent laboratory can advise on any specific fixation technique required before euthanizing the animals or doing the necropsy.

In the case of companion pet chickens, taking whole-body ventrodorsal and laterolateral radiographs of the carcass can help to identify among other, fractures, ascites, pneumonia, air sacculitis, internal hemorrhages, organomegaly (spleen, liver, kidneys, heart), swelling, foreign bodies, neoplasia, granulomas, and intestinal dilatation.

14.5 Whole Carcass Conservation

The weight and the body condition score of the dead chick/s should be determined as soon as possible since postmortem changes may modify weight measurements. Carcasses that cannot be examined immediately should be wetted (with water and detergent), placed in single or double plastic leak-proof bags, and properly labeled until necropsy. This procedure will allow for adequate refrigeration of the specimen for the next hours, shipping, and transport to facilities or to the laboratory. Wetting the carcass with a disinfectant also prevents the dispersal of feather dust and feces and inhibits or kills potential pathogens. Spraying and submerging the whole carcasses (except the head) in disinfectant are the most common approaches. Freezing carcasses will impair histopathological examination of tissues and organs, and it is not usually recommended. Freezing the carcasses, however, will minimally impact toxicological and microbiological investigations.

14.6 Necropsy Protocol

14.6.1 First Thing First

Several necropsy protocol forms and instructional videos are available online. Experienced avian/poultry veterinarians may develop their own necropsy protocols and data collection forms. While the order of different steps may vary because of each veterinarian's preferences, in any case examination of the carcass should follow a consistent approach. Repeated deployment of the same necropsy protocol and practice will result in developing the ability to identify both normal and abnormal macroscopic changes.

Chicken breed, sex, age, and individual identification (number on wing/leg bands, pens, coops, cages, and pastures in organic chicken farming) are important baseline information elements and should be properly recorded.

When in doubt about the breed, taking pictures before euthanizing the animal or before the necropsy starts is strongly recommended; this will allow definitive identification *a posteriori*. Becoming familiar with different breed standards, use, characteristics, and predisposition to diseases is mandatory for the poultry veterinarian. The same is true about the different commercial poultry breeding approaches (layers, broilers, exhibition, breeders, pets). Some breeds may present features that could be abnormal in other breeds (e.g. supernumerary toes, black skin, and silky feathers in Silky Chinese chickens). Understanding chicken production systems, biosafety measures, prophylaxis, husbandry, and other management aspects are important and are discussed elsewhere.

TIPS!

Before necropsy starts, it is helpful to perform a complete physical examination on the carcass as it is done on the live bird.

Photographs can be taken in between steps, either by the veterinarian performing the necropsy or by an assistant.

Biosecurity measures should be taken as previously indicated.

Saving carcasses post necropsy is recommended, as they can provide additional information if the original investigation did not yield results or answers, or additional tissue needs to be evaluated.

Adequately disinfect and clean all reusable instruments used during the necropsy.

Collect sterile samples for cytology and microbiology for culture and molecular diagnosis from representative sections of both normal and abnormal tissues for every organ and tissue.

14.6.2 External Examination

1) Evaluate rhinotheca and gnathotheca, as well as the beak joint and commissures, for external abnormalities, flaky keratin, hyperkeratosis, fractures, cracks, discoloration, overgrowth, and "scissor" beak. Evaluate ear opening for exudates, parasites, and developmental anomalies (Figure 14.2A).
2) In case of sinusitis, nasal secretion, and enlarged sinuses, incise aseptically the skin over the infraorbital sinus to collect sterile samples for cytology and microbiology (culture and molecular diagnosis) (Figure 14.2B).
3) Inspect the oral cavity, palate, choanal slit, oropharynx, tongue, and palatal spines. Evaluate changes in color and for the presence of masses, wounds, plaques, ulcers, and diphtheritic membranes. In fresh, recently dead, or euthanized specimens, describe the color of non-pigmented mucosa. Collect saliva and other secretions for cytology, culture, molecular diagnosis, and parasites detection (Figure 14.2C).
4) Examine the head for trauma, skin lesions, and neoplasia, and the wattles and barbs for edema, ulcers, scabs, congestion, and hemorrhages. Save samples of these tissues for diagnostic purposes. The infraorbital sinus can be cut open by a frontal plane section just in front of the eyeballs (orbit). Examine the turbinates for the presence of exudates, abnormal growth, parasites, and other abnormalities. Ear canals can be examined for detritus and exudates. Remove the skin and expose the skull and the foramen magnum.
5) The eyeballs keep the intraocular pressure for a short period of time once the chicken is dead, so a detailed ophthalmoscopic examination of the anterior and posterior chambers, retina, optic papilla, and pecten should be performed ideally pre-mortem or immediately after. Nevertheless, inspect at least the cornea, sclera, eyelids, nictitating membrane, and lens. Take appropriate samples in case of conjunctivitis (usually associated with sinusitis). The globe can be removed and fixed. Inspect the orbital space (Figure 14.2D).
6) Palpate the neck, esophagus, and crop to collect samples from the content (food, fluids, regurgitated material). Evaluate subcutis for the presence of emphysema. Evaluate crop and esophagus for masses, exudates, and ulcers. Inspect the apteric area of the skin and jugular vein on both sides of the neck.
7) Transillumination of the trachea through the skin with a flashlight or a Finoff transilluminator will allow the detection of tracheal worms (e.g. Gapeworms: *Syngamus trachea*) or masses (e.g. Aspergillomas).
8) Palpate pectoral muscles to determine body condition score and the presence of unilateral or bilateral muscular atrophy. Atrophy suggests a neurological issue or a traumatic injury to the ipsilateral limb or, if bilateral, generalized malaise. Other abnormalities in this area are lack of or reduced subcutaneous fat, keel fractures or abnormalities, subcutaneous edema, and skin ulcerations at the points of contact with the keel (e.g. as a result of paresis/paralysis).
9) Inspect the vent. Lack of tone is a common post-mortem finding. Look for signs of diarrhea (dirty appearance or soiling of feathers around the cloaca). Collect cloacal feces for microbiology and endoparasite investigation. Collect gastrointestinal content for additional endoparasite investigation (Figure 14.2E).
10) Examine both legs and wings for fractures, luxation, muscular atrophy, swelling of joints, hemorrhages,

Figure 14.2 External examination of the head. (A) Secretion associated with the nares and beak. (B) The infraorbital sinus is exposed. (C) Observation of the oral cavity (mouth). (D) Examination of the eye and associated structures (conjunctiva and conjunctival sac). (E) Vent and external cloaca. Examination of the mucosa and associated structures to detect blood, feces, mites, ulcers, papilloma, etc. (F) Evaluation of the legs for anomalies (edema, hemorrhages, ulcers, and hyperkeratosis) and examination of the nails.

edemas, abscesses, and any other abnormalities. Evaluate mobility, range of motion, presence of swelling, luxations, and possible fractures of the shoulder, elbow, or carpal joint in the wings and hip, knee, hock joints, and toes in the hindlimbs.

11) Palpate the coracoid, clavicular, and scapular bones for potential fractures or luxation. Examine the interclavicular air sac for air sacculitis or the presence of granulomas.
12) Examine the feathers (primaries, secondaries, rectrices, body feathers) for the presence of stress bars, ectoparasites (lice, fleas, Hippoboscidae flies, mites), abnormal pigmentation, blood feathers, pinched-off feathers, abnormal molting sequences, cysts, and retained and/or missing feathers. Inspect feathers in vain with the help of a magnifying lens or a microscope. Look for developmental abnormalities such as silky feathers (which are normal in certain breeds). Collect feathers and skin samples if needed and quantify ectoparasites infestation as mild, moderate, or severe according to the number observed. Collect parasites for further identification. Take samples from different affected body regions. Abnormal feathers and follicles may be saved in formalin solution for further evaluation or refrigerated for microbiology.
13) Examine the skin and subcutaneous area for the presence of hematomas, wounds, infection, abscesses, neoplasia, and emphysema. Iatrogenic hematomas may result from euthanasia (intravenous injections of barbiturates and/or atlantooccipital dislocation), fractures, and coagulation problems caused by direct or indirect ingestion of anticoagulant rodenticides. Depending on the time passed since the death or euthanasia, skin turgor, and elasticity can be used to assess the bird's hydration status. It is not uncommon to find larvae (maggots) of several species of flies (Order Insecta, Family Diptera) on the skin associated with wounds, dermatitis, or diarrhea (cloacal area).
14) Place the chicken in ventral recumbency and wet the spinal area with alcohol. Search for fractures, hematomas, and any other lesion along the vertebral (spinal) axis, including the neck and pygostyle.

Head-neck luxation resulting from euthanasia procedure may be accompanied by hematoma.

15) Evaluate the legs for scale anomalies, edema, hemorrhages, ulcers, and hyperkeratosis (as a result of *Knemidocoptes* spp. mite infestation, Poxvirus or hypovitaminosis A), pododermatitis and abrasions caused by irritants (highly concentrated disinfectants, wet soils). Examine the nails and, in the case of males, the spurs for overgrowth, deformation, and trauma. Short spur may be present in females of certain breeds (Figure 14.2F).
16) Examine the cloaca, cloacal mucosa, and associated structures by gently reverting the mucosa with swabs. Inspect the cloacal sphincter for dermatitis, ulcers, scabs, soiled feathers, discolored feces, hematochezia, mites, and any other sign of enteritis/cloacitis, retained eggs, uroliths, and mucosal papilloma (Figure 14.2E).

TIPS!

Remember to annotate necropsy findings after each step, even if no lesions or pathological changes were noted.

Be sure at this point that all external investigation of the chicken is completed. If in doubt, repeat the examination of any specific region and collect additional samples that may be needed later.

For the internal examination of the chickens, changing gloves between sample collection may be essential for the accurate etiological diagnosis of infectious disease, thus preventing the carryover of nucleic acids. Molecular diagnostic techniques are very sensitive and capable of detecting even small amounts of DNA/RNA.

14.6.3 Internal Examination

14.6.3.1 Subcutaneous and Muscles

1) Dislocate both coxofemoral joints to place the bird in dorsal recumbency and provide stability during the following procedures.
2) Incise the skin along the midline of the bird, starting at the naked skin area behind the gnathotheca through the body. Stop a few millimeters before the cloaca. In the neck, be mindful of underlying structures, such as the esophagus and crop, and at linea alba be mindful of the celomic cavity as well. The subcutaneous space may be filled with fat in healthy or overweight birds, but it could be a small space that directly contacts the linea alba in sick, debilitated, or cachectic chickens. Therefore, it is wise to be careful to avoid accidental incision of the celomic cavity during this step. A grooved probe or the space between forceps to guide the scalpel can prevent accidental incisions into the celomic cavity.
3) Gently reflect the skin of the neck, breast, wings, and legs laterally with the help of the blunt side of a scalpel handle or forceps, separating it from the proximal wings, legs, and the body. Evaluate pectoral, supracoracoideus, leg, wing, and abdominal muscles. In chickens, the breast muscles are paler than wing or leg musculature (Figure 14.3A).
4) Evaluate the subcutaneous space for discoloration, fluids, hematomas, adherences, and subcutaneous masses. Note the characteristics of any subcutaneous adipose tissue, which is usually yellow to yellowish orange in color depending on the presence of carotenes in the diet.
5) Along the lateral surface of the neck, the jugular veins are clearly visible. Examine the shoulder and coxofemoral joints (previously dislocated) (Figure 14.3B). Evaluate the color and integrity of the cartilage. Subcutaneous fat deposits are also visible at the thoracic girdle. If needed, additional examination of the clavicle and coracoid bones, and interclavicular air sac can be performed.
6) In case of confirmed or suspected air sacculitis, pneumonia, or other respiratory conditions, air sacs can be sampled for microbiology (culture and molecular diagnosis) before accessing the celomic and thoracic cavities, thus reducing the risk of external contamination. A small entry access into the caudal air sacs can be performed using sterile, nucleic acid-free, curved, Halstead forceps, between or behind the last two ribs, as for laparoscopy, by pushing the tip of the forceps through the abdominal oblique muscles. With the Halstead forceps in place and inside the air sac, gently open its arms to facilitate the insertion (avoid touching the arms of the forceps) of a swab or a catheter. Once inside, either by swabbing or washing the air sac space with sterile saline solution through the catheter, a microbiology sample can be obtained with minimal disruption or contamination. The interclavicular air sac can also be easily accessed by making an incision on the skin covering the interclavicular space.
7) At this point, the trachea, esophagus, and the crop are clearly visible. A swab can be inserted into the esophagus and the crop to better visualize them. The thymus glands can be observed along the neck.
8) Incise the pectoral muscles along and parallel on both sides of the carina (keel) and bluntly dissect the muscles separating them from the sternum, furculae, and coracoids. Examine both pectoral and supracoracoideus muscles for symmetry, paleness, discoloration

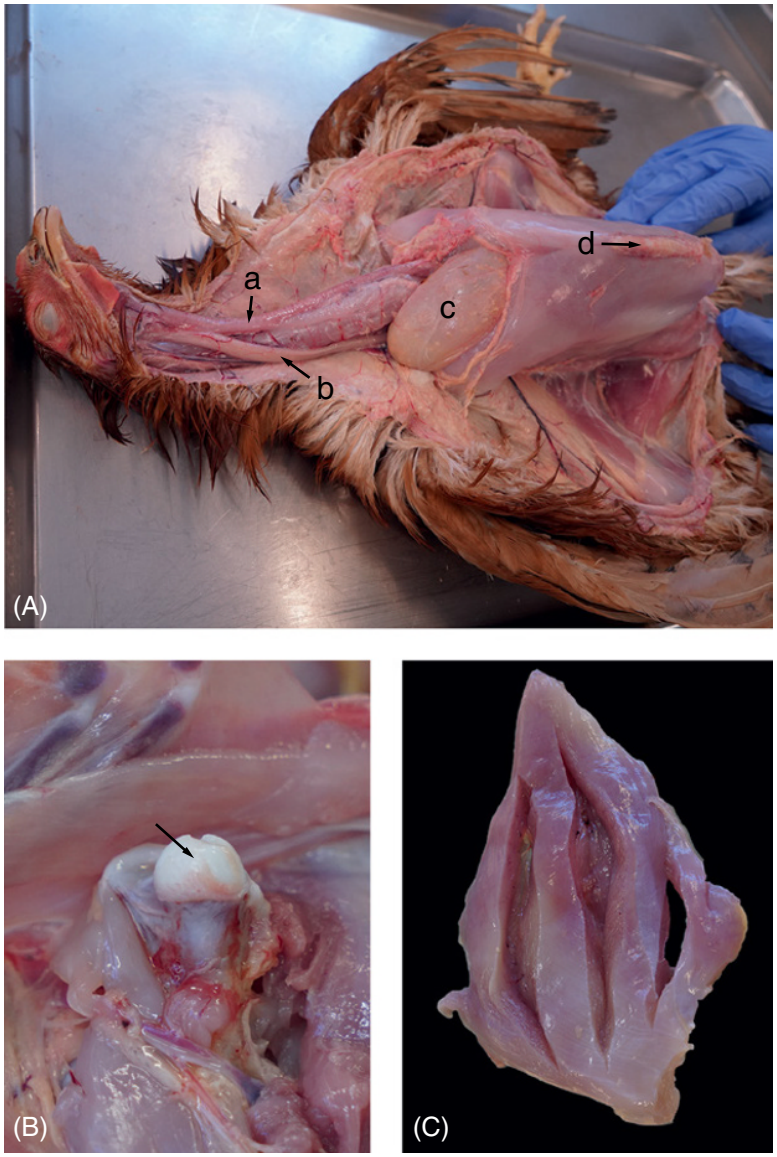

Figure 14.3 (A) Subcutaneous observation of structures and lesions. (a) Trachea, (b) esophagus, (c) crop, and (d) sternum (keel). (B) The arrow shows the color and integrity of the cartilage of the femoral head (dislocated). (C) Parallel incisions on the dissected pectoral muscles to evaluate for lesions.

(e.g. white colored: white stripping disease; green colored: deep pectoral myopathy), presence of cysts, parasites, or hematomas (Figure 14.3C). When approximating the shoulder joint space, carefully dissect this area to assess the extra thoracic extension of the interclavicular air sac. Healthy air sac extensions should be transparent and avascular. When an infectious respiratory disease is present (e.g. aspergillosis, bacterial pneumonia), changes in the air sac extension could be the first indication of this condition during necropsy. Nevertheless, a normal extra thoracic extension of the interclavicular air sac does not preclude these conditions.

14.6.3.2 Celomic Cavities

Access to the celomic cavities (abdominal and thoracic) is usually done simultaneously. Make a small poke incision in the linea alba just behind the caudal midline edge of the keel (Figure 14.4A–C). Extend the incision to both sides of the sternum. Using straight sharp blunt scissors, bandage removal scissor, or chicken shears (this will depend upon age, size, and overall calcification and size of ribs and thoracic girdle bones of the bird) cut the ribs at the level of the costochondral joints until reaching the thoracic girdle. Cut the coracoid and furcula on each side. When cutting the ribs, be mindful of the underlying air sacs and avoid cutting them by keeping the internal shaft of the scissor

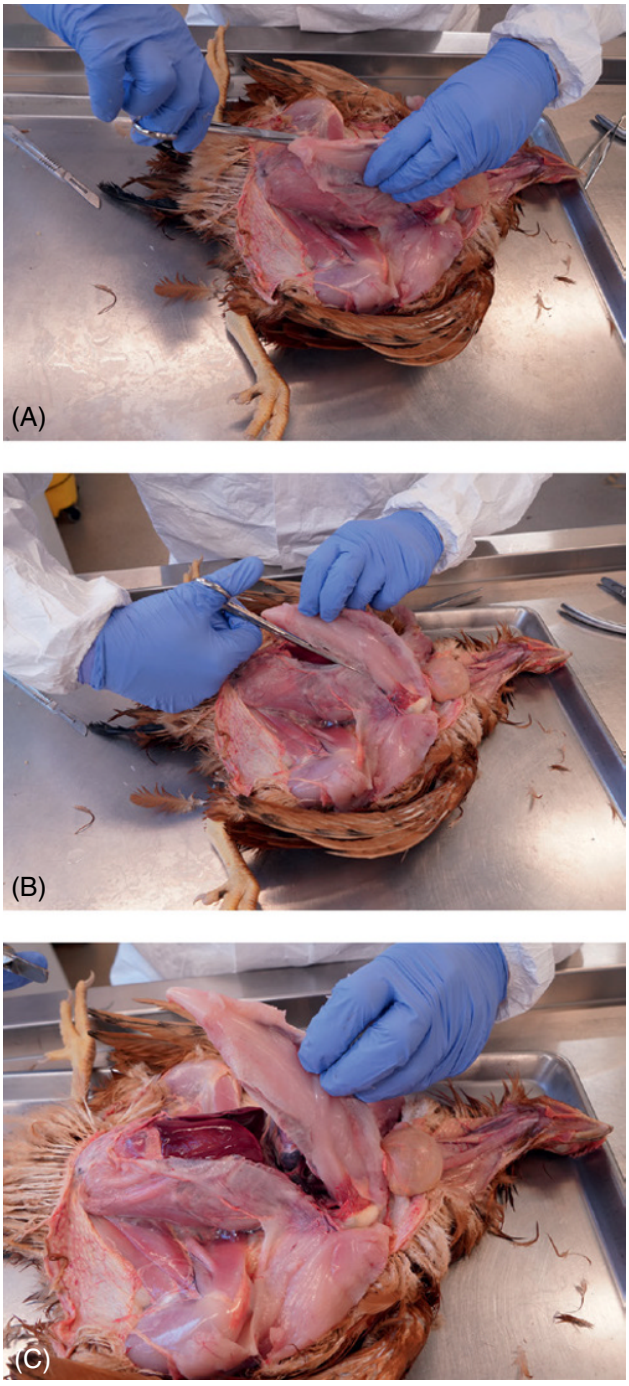

Figure 14.4 Demonstration on how to access the celomic cavity. (A) A small incision is performed in the linea alba just behind the caudal midline edge of the keel. (B) The incision is extended to both sides of the sternum. (C) The ribs are cut at the level of the costochondral joints until reaching the thoracic girdle. The coracoid and furcula are also cut on each side.

close to the internal wall (Figure 14.4B). Carefully cut any connective or muscular tissue between the clavicle and sternum that attaches to the ligaments connecting the pericardial sac and liver followed by carefully removing the sternum (Figure 14.4C). The thoracic cavity is now fully exposed and shows the intrathoracic esophagus, heart, brachiocephalic trunk, thymus, thyroid and parathyroid glands, lungs, and air sacs.

Collecting samples for culture and molecular diagnosis at this time could be deceiving, due to contamination from previous steps. Blood and any fluid identified in the celomic cavities that are not due to necropsy can be aspirated and processed before opening the celoma. If fluids in the celomic cavities are due to the access to this cavity during the necropsy, use sterile gauze or swabs gently to remove any excess fluid. This will clean the work field and allow for the inspection of the kidneys, ureters, adrenal glands, gonads (ovaries and testicles), deferent ducts (male), and oviduct (hen) without causing additional contamination. It is noteworthy to mention that microbiological investigation of these structures requires different approaches that are beyond the scope of this chapter.

TIPS!

Remember to use different sets of instruments to manipulate and collect samples from air sacs, lungs, liver, spleen, and any other organ for the molecular investigation of infectious agents.

Additional microbiology sampling for culture and smears can be collected using sterile swabs as indicated previously.

When removing organs, place them on clean, sterile surfaces or at least on clean paper towels to avoid cross-contamination.

Carefully and consistently observe and identify different organs exposed by the removal of the sternum and thoroughly assess the following:

14.6.3.3 Heart

Describe its position, size, color, and shape. A small amount of clear, transparent fluid in the pericardial space is considered normal; collecting and analyzing this or any abnormal fluid present in this pericardial space is recommended. Identify changes in the pericardial sac, such as opacity, urate precipitates (*note: intracardiac injections of barbiturates will cause salt precipitates resembling urate deposits as in gout*), adhesions, and masses on serosal surface of large vessels. If serum is needed for serological diagnosis, small amounts of serosanguineous fluid can be aspirated from the heart chambers. Remove the heart, with an intact pericardial sac, and the brachiocephalic trunk for further examination by gently holding the brachiocephalic trunk and cutting with small scissors all the connections to the main vessels. Use sterile swabs and gauze to absorb blood

or serum spilled during this step. Blood clots in the auricles and inside larger vessels can be found, and serosanguineous fluids could come out when cut.

With the heart and larger vessels removed, carefully cut the apex of the heart transversally using Iris scissors. Observe the differences between the thickness of the left and right ventricular walls. Cut throughout the ventricles and auricles on both sides, exposing the papillary muscles, valves, tendinous cords, and both right and left atria. Examine these structures and the endothelium of major vessels for content, wall thickness, heart valve fibrosis, and ruptured tendinous cords (Figure 14.5A–C).

14.6.3.4 Liver and Gall Bladder

Assess the liver and evaluate its position, size, color, shape, borders, and any changes of the parenchyma, such as discoloration, foci of necrosis, masses, opaque surface, rounded borders, and cysts (Figure 14.6). Cirrhosis will manifest as a reduction in size, with fibrous tissue replacing the normal parenchyma causing the shrinking of the organ. Amyloid deposits will cause color changes in the parenchyma to orange or bronze and give a sandy, non-friable, appearance to the liver when incised. The liver and the spleen (see below) are important immunological organs; focal or multifocal yellow foci of inflammation and/or necrosis are

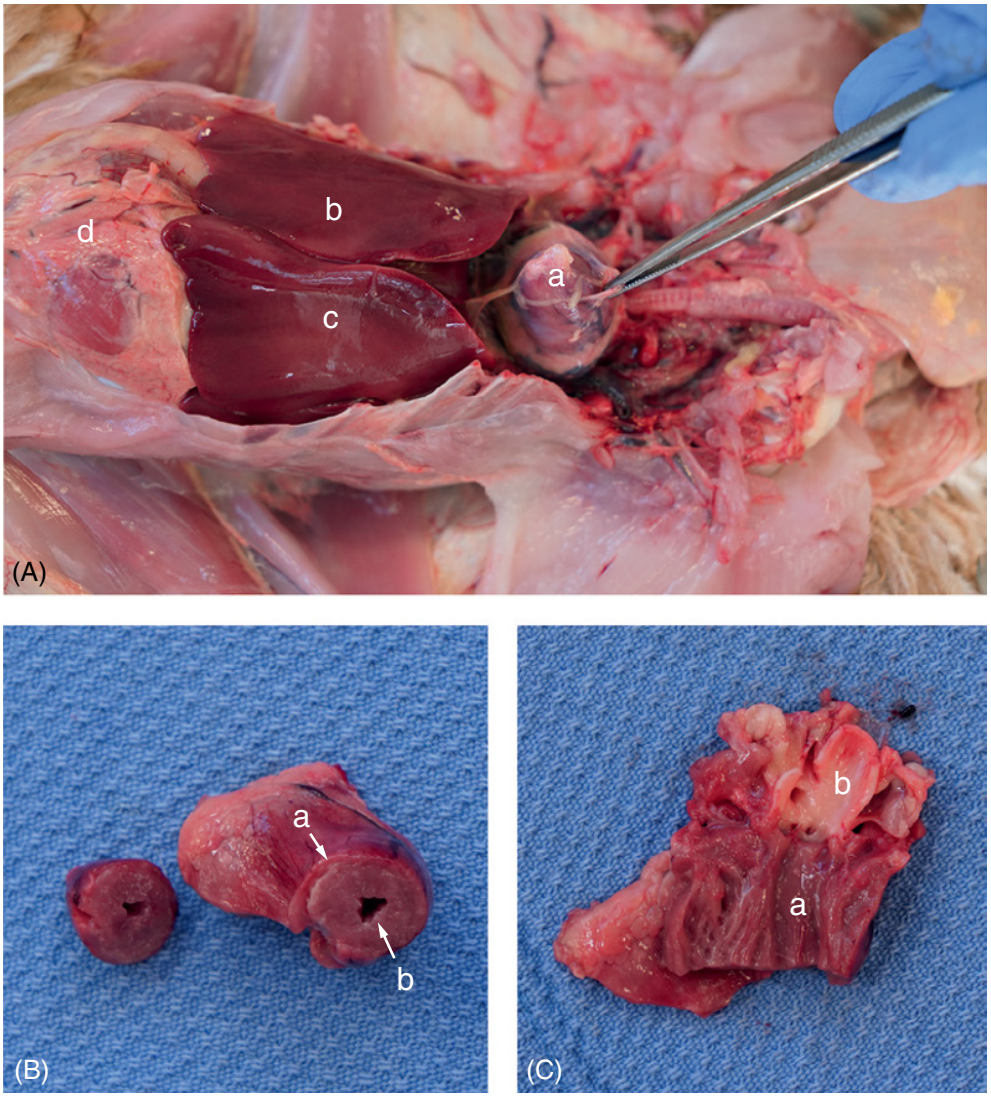

Figure 14.5 (A) The heart is identified inside the pericardial sac (a), in close contact with the right (b) and left (c) lobes of the liver. Caudal to those, the gizzard can be observed (d). (B) Once the heart is removed, the apex of the heart is transversally sectioned. The differences between the thickness of the right (a) and left (b) ventricular walls are evident. (C) The ventricles and auricles on both sides are cut, exposing the papillary muscles (a, left ventricle), valves, tendinous cords, and main vessels (b, aorta).

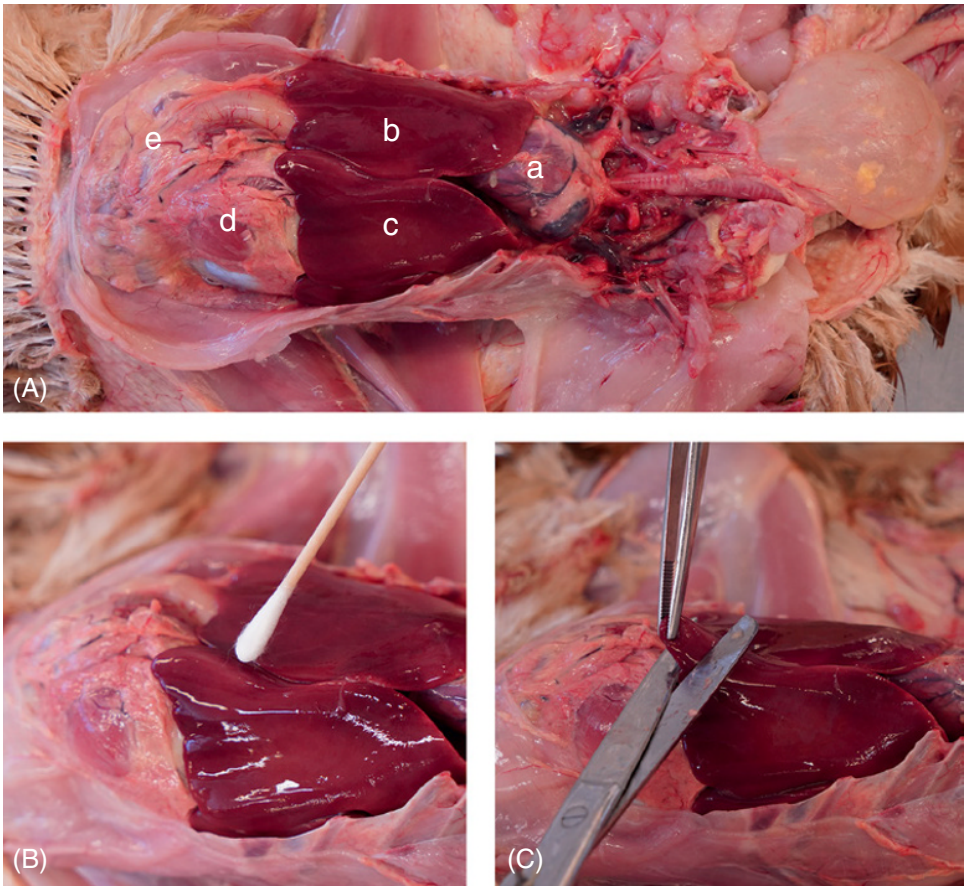

Figure 14.6 (A) The liver is evaluated for position (caudal to the heart (a)), size, color, shape, borders, and any changes of the parenchyma, such as discoloration, foci of necrosis, masses, opaque surface, rounded borders, and cysts. (b) Right and (c) left liver lobes. (d) Gizzard (ventriculus), (e) small intestine. (B) Swab to obtain microbiological samples for culture and/or molecular diagnostics of bacteria. (C) Tissue sample for histopathology, molecular microbiology and culture, or toxicology testing.

common findings with many bacterial and viral infections. Most infectious diseases will cause changes in these two parenchymatous organs; hence, the liver and the spleen can be ideal sites for microbiological investigation. Be sure to take appropriate samples for culture and/or molecular diagnostics of bacteria (anaerobic, aerobic, mycobacteria, Mycoplasma spp., and Chlamydia spp.), fungi, and viruses.

Gently remove the liver with the associated gall bladder, cutting all ligaments and bile ducts before ending in the duodenum. Chickens and other galliformes have a gall bladder, a small, rounded sac characterized by its dark emerald-green color. The gall bladder is attached to the liver, so be careful when removing it, as it can easily break or be punctured. The bile ducts drain into the duodenum, and organs in contact with the gall bladder may be stained due to their proximity.

14.6.3.5 Air Sacs

Examine the cranial and caudal thoracic and interclavicular air sacs. Any alteration of the normal appearance of the air sacs should be considered pathological. In chronic granulomatous infections of the air sacs, large, inspissated caseous material may fill the air sac spaces. Fibrin and caseous plaques can be caused by several species of bacteria and green velvet, moldy appearance is commonly observed in aspergillosis. Neovascularization (remember, air sacs are non-vascular structures) accompanied by fluids, plaques on the air sac walls, and cloudiness are all indicative of inflammation and usually reflect on an infectious etiology. Abdominal air sac can be accessed. Using Adson tissue forceps elevate the abdominal muscles and incise them through the linea alba. Reflect the abdominal muscles to both sides by extending the midline incision as done cranially and examine the celomic cavities. The celomic cavities will have an adipose tissue pad filling up the space. Save a sample at this point if any sign of steatis or fat necrosis is present. Remove the fat avoiding touching other internal celomic organs.

Collect samples from the abdominal air sac if they are still intact or from the celomic cavity (peritoneal portion) if the abdominal sac walls were already incised.

14.6.3.6 Thyroid and Parathyroid Glands

Both glands are found together close to the common carotid artery and the brachiocephalic trunk. In most cases, only the thyroid can easily be macroscopically differentiated, due to its size and dark red color. When visible, parathyroid glands are yellowish in color.

14.6.3.7 Proventriculus

This organ is usually covered by the bilobed liver. It will become more visible once the liver and heart are removed, and when extracted in block together with other gastrointestinal system organs (see below) (Figure 4.10 and Figure 14.7).

Variable amounts of fat may be present in the thorax. Besides the organs listed above, pay attention to the serosal surfaces and large vessels for enlargements, plaques or growing masses on the serosal surfaces, and changes in their thickness.

14.6.3.8 Lungs

Once the heart and liver are removed, the lungs are exposed. This is the most convenient time to collect samples from the lungs for culture and molecular diagnostics of infectious diseases. Asses the organ for discoloration, granulomas, hemorrhages, neoplasia, edema, necrosis, and congestion.

The mandibular bones, the tongue, and the tracheal opening can be separated from the maxillary bones and skull with strong scissors. The trachea will remain behind the tongue and can be easily dissected from the neck and esophagus connections. Carefully blunt dissect the syrinx, primary bronchi, and lungs. The latest can be easily removed by blunt dissection using a scalpel handle or forceps, separating the lungs from their attachment to the ribs. Avian lungs are not collapsible and maintain their size and shape once removed from the open thorax. Rib impressions on the lungs can be noticed upon removal of these organs from the rib cage. Examine the lung surface, ostium, and primary bronchi. Make several incisions through the lung parenchyma looking for lesions and save several sections for histopathology (Figure 14.8A).

Using Iris or small, straight, blunt/sharp scissors prepared for microbiological investigation cut the trachea along two lines corresponding to its lateral sides in two halves (Figure 14.8B). Examine the tracheal mucosa (Figure 14.8C). Culture and molecular investigation of upper respiratory tract infections is possible, although postmortem contamination with oral saliva, gastrointestinal reflux, water, or disinfectants would undermine the diagnostic value of these samples. Tracheitis, granulomas, parasites, and adhesions are potential findings in chickens. Examine the syrinx and the primary bronchi as indicated for the trachea. Place these small organs or their sections in cassettes for processing. Pay attention to the presence of masses, exudates, and mucosal lesions in the collected samples as needed.

14.6.3.9 Spleen

The spleen lies in between the ventriculus and proventriculus and is slightly located on the left side of the celomic cavity. To visualize it *in situ*, rotate the ventriculus and proventriculus counterclockwise. Note the size, shape, and color of this organ and dissect it for later examination. The spleen also could be collected with the proventriculus and ventriculus if not removed at this stage. Splenomegaly, necrosis, and inflammatory foci are common findings in chickens with systemic infections, such as avian mycobacteriosis, and viruses such as fowl adenovirus, chicken

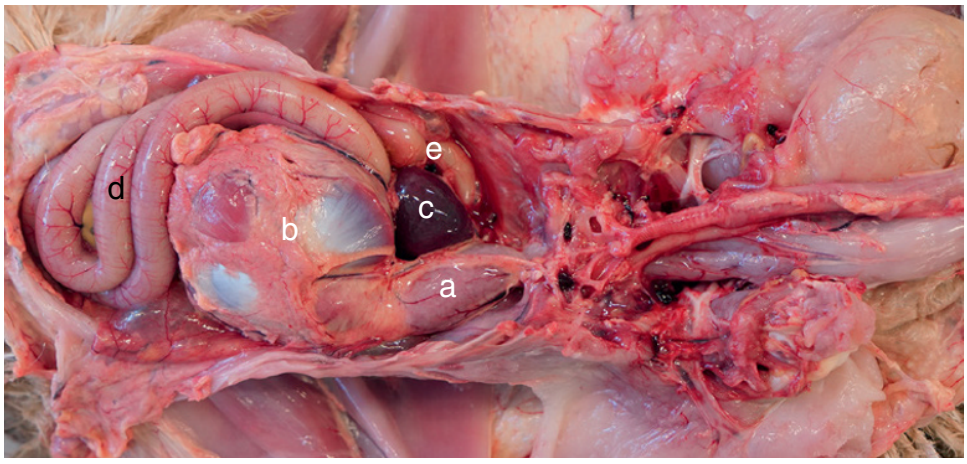

Figure 14.7 Once the liver and heart are removed, the proventriculus (a), gizzard (b), spleen (c), small intestines (d), and the tip of the ceca (e) can be observed.

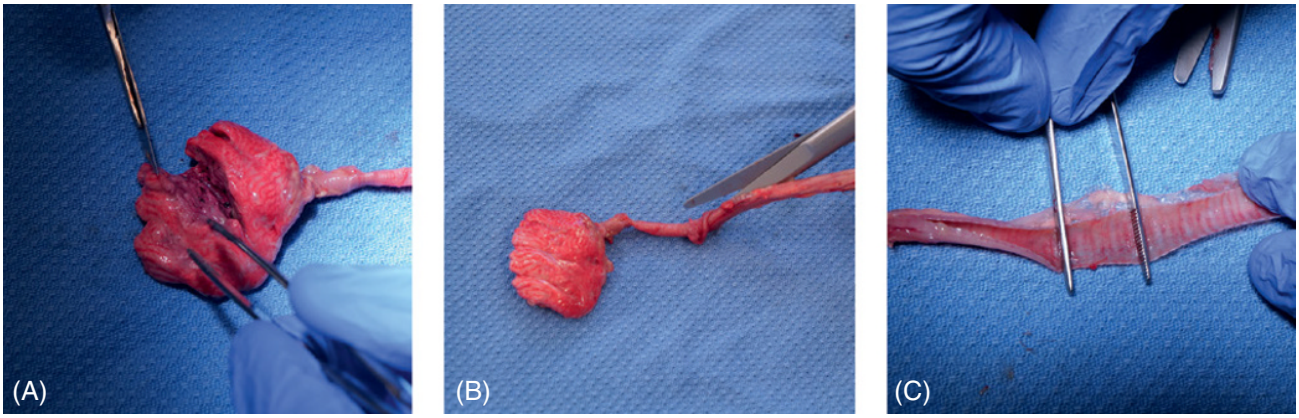

Figure 14.8 (A) Once the lung is dissected from the thoracic cavity, several incisions through the lung parenchyma are made to look for lesions and save several sections for histopathology and toxicology. (B) The trachea is cut using small, straight, blunt/sharp scissors. (C) Examination of the tracheal mucosa.

anemia virus, and reticuloendotheliosis virus. Sometimes, lesions can replace most of the organ parenchyma. Paleness of the spleen usually indicates anemia. Hemangiosarcoma, although rare, can be seen in chickens.

TIPS!

Small organs such as the thymus, bursa of Fabricius, thyroid and parathyroid glands, air sacs, deferent ducts, and uropygial gland can be easily overlooked during necropsy.

Once removed and after investigating each organ, save them immediately in formaldehyde.

Be mindful of placing these organs and thin sections of larger organs such as the liver, spleen, and lungs, in histological cassettes with appropriate labeling.

14.6.3.10 Gastrointestinal Tract

Using clamps or forceps, clamp the proximal end of the esophagus caudal to the oropharynx. Do the same with the distal colon cranial to the cloaca. Alternatively, you can use thread to ligate both ends of the gastrointestinal tract. Gently and bluntly dissect the connective tissues and ligaments to remove the complete gastrointestinal tract including the esophagus, crop, proventriculus, ventriculus (gizzard), and small and large intestines (Figure 14.9A and B).

Examine the gastrointestinal tract externally for lesions, changes in color, masses, enlargements, areas of discoloration and necrosis, and congestion of the serosa. Palpate the organs thoroughly, paying special attention to the thickness of the intestinal wall. Examine the intestinal serosa for masses, congestion, hemorrhage, or necrosis.

Proceed to examine the content of each of the organs. By using delicate scissors such as Iris scissors, start cutting the intestines from the distal end and examining its content and mucosa (Figure 14.9C). Evaluate all organs for wall texture and thickness, presence of hematochezia or abnormally colored feces, undigested food, ulcers, necrosis, foreign bodies, or any other lesion in the mucosa. Macroparasites and microparasites can be investigated using traditional parasitological techniques. In the proventriculus, examine the mucosa for ulcers, blood, parasites, and any other significant lesion. In the ventriculus, investigate the presence of food, grit, parasites, foreign bodies, masses, and blood. Evaluate the isthmus between the two stomachs, which can be easily identified by the smooth surface and lack of glands.

When examining the ventriculus use Mayo or blunt/sharp scissors to open it. Collect grit and food and evaluate for the presence of parasites by gently washing the proventriculus and ventriculus and collect the wash on Petri dishes. The kaolin layer from the ventriculus can be removed using Adson forceps; this will allow examination of the underlying mucosa.

When examining the intestinal lumen, identify the pancreas attached in between the duodenal loops. When separating the gastrointestinal system *en masse*, identify the pancreas and set it aside for examination. Examine the duodenum, followed by the jejunum, ileum, the ileo-cecocolic junction, bilateral ceca, and colon. Ulcers, hemorrhages, necrosis, raised erythematous lesions, hematochezia, and pseudomembranous colitis are common findings because of coccidial, bacterial, and viral infections.

TIPS!

If toxicosis is suspected, crop, proventriculus, ventriculus, and intestinal content should be saved and frozen for further investigation. The liver, kidneys, brain, and bones are also useful for detection of neurotoxins, anticoagulant rodenticides, heavy metals, and other toxins.

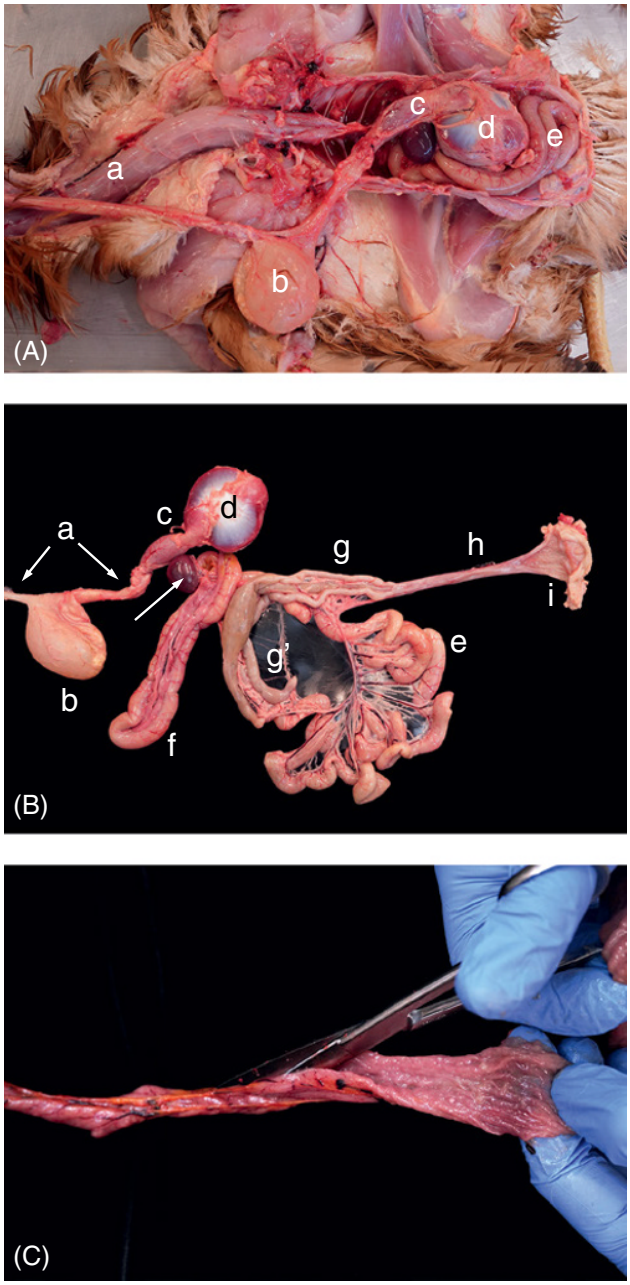

Figure 14.9 Gastrointestinal tract *in situ* (A) and *ex situ* (B). (a) Esophagus, (b) crop, (c) proventriculus, (d) gizzard (ventriculus), (e) jejunum, (f) duodenum with pancreas inside the loop, (g) base of the ceca, (g′) apices of the ceca, (h) colorectum, and (i) cloaca. The single arrow points to the spleen. (C) Procedure to examine the content and mucosa of the intestines cutting with scissors from the distal end.

14.6.3.11 Pancreas

Changes in coloration, enlargement, congestion, necrosis, and edema could be indicative of pancreatitis. Pancreatic tumors are rare in chickens (Figure 14.9B).

14.6.3.12 Gonads and Associated Organs

Gently dissect the gonads and the adrenal glands together, which are located ventrocranially to the kidneys. Both testicles are present; they are white ivory colored, sometimes pigmented (black or dark brown, depending on the breed), oval, and with a smooth surface. Testicles can be as small as a few millimeters at hatching time to increase their size to several centimeters in sexually active males.

Both left and right ovaries develop at the early stage of development in chicken, though, only the left reaches its full development and contributes to the ova production while the right ovary involutes along with the right oviduct leaving a remnant right oviduct close to the cloaca.

In laying hens, the left oviduct can be easily identified, especially in sexually mature (egg-laying) hens. Laying hens may have multiple follicles at different stages of maturation. Sick and emaciated hens may show limited gonadal activity, although sometimes ova can still be observed. The oviduct can also be inactive or active, and its size can range from almost nondetectable in young chickens to occupying a large part of the celomic cavity in adult, egg-laying hens. Once removed, identify its different parts (infundibulum, magnum, isthmus, uterus, and vagina). Gently open it up along its whole length to examine the mucosa and save appropriate small sections for histopathology. The thickness of the oviduct can vary depending on the age, breed, and sexual maturity and activity of the birds (Figure 14.10).

Culturing the oviduct at this stage is possible if it is removed aseptically, sterilizing the surface, and swabbing the lumen through an incision at different points or by aspirating the lumen with a small gauge needle attached to a syringe.

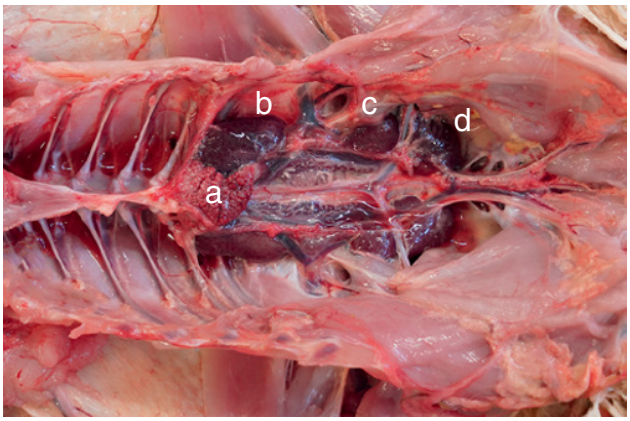

Figure 14.10 Body of a young hen once the digestive system has been removed. The immature left ovary (a) is observed associated with the cranial division of the kidneys (b). The vessels of the renal portal system divide the middle (c) and caudal (d) renal divisions.

14.6.3.13 Kidneys and Adrenal Glands

Kidneys and adrenal glands are visible once the other intracelomic organs have been removed. In birds, the kidneys are not fully lobated but partially divided into cranial, middle, and caudal divisions (Figure 14.10). They can be easily identified at this point of dissection. They are found lying on the synsacrum fossa. Evaluate their color, shape, size, texture, subcapsular fluid accumulation, and the presence of masses or discoloration. Ureters run ventral to the kidneys and will be revealed by the presence of white urates inside. Retained urates in the kidney could be a sign of kidney disease, gout, or dehydration, but also can be a common post-mortem finding, especially in moribund birds.

Carefully dissect and remove the very friable kidneys using tissue forceps. Underneath the kidneys, the lumbosacral plexus including the sciatic and other nerves can be now visualized. Evaluate the size and shape of these nerves and plexus, excise them, and place them in histopathological cassettes. Marek's disease usually causes a notorious enlargement of the sciatic nerve by virion infiltration.

With the kidneys, gonads, and adrenal glands removed, check for any abnormalities in the celomic peritoneal cavity, the pelvic bones, spine, and other visible structures like nerves.

14.6.3.14 Bursa of Fabricius

Using scissors, cut around the cloaca and the cloacal opening and remove it from the bird body. In young chicks, the bursa of Fabricius (easily identified when present) lays dorsal to the cloaca (Figure 11.3).

14.6.4 Bones and Bone Marrow

Bones and bone marrow can be examined once other organs have been evaluated. Bone marrow can be collected from the ulna and tibiotarsus by breaking these bones carefully with forceps or bone cutters (Roengeurs) and placing them immediately in formaldehyde solution (Figure 14.11). Fine needle aspiration of bone marrow should provide enough material for microbiological investigation. Samples can also be collected by carefully breaking these bones with instruments free of nucleic acids.

14.6.5 Uropygial Gland

Evaluate the uropygial gland on the dorsal side of the tail and pygostyle. Carefully cut the feathers and dissect the skin to isolate the adjacent uropygial gland. Assess function by gently compression and save it in formaldehyde solution.

Figure 14.11 Bones and bone marrow (arrows) can be examined once other organs have been evaluated. Bone marrow can be collected from the ulna and tibiotarsus by breaking these bones carefully with forceps.

14.6.6 Skull

Examine the skull, which was previously separated from the neck. Using Mayo scissors or a saw, open the skull through the foramen magnum by cutting the calvarium, making a semicircle, through the occipital, squamosal, parietal, and frontal bones. Once the cranium is open, the brain can be removed from the skull by gravity and gentle dissection. Make several sagittal and along the midline cuts. Malacia, edema, congestion, hemorrhages, abscess, necrosis, atrophy, and aplasia/hypoplasia are potential lesions commonly observed in chickens with neurological signs. Alternatively, the open skull can be submerged in formaldehyde solution, and once the brain is fully fixed it can be easily removed. Although this procedure better preserves the integrity of this organ, this approach excludes gross examination of the brain.

14.6.7 Vertebral Column and Spinal Cord

Sections of the vertebral column can be fixed, decalcified, and processed for histopathology. The spinal cord can be fixed, processed, and evaluated by histopathology. Postmortem changes occur rapidly in the spinal cord and gross sections can be examined macroscopically, especially if neurological disorders associated with this organ were reported. Lesions to the spinal cord can be caused by infectious agents, parasites, tumors, neoplasia, trauma, or degeneration. Collection of cerebrospinal fluid is rarely attempted in birds.

Further Reading

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15

The Egg Anatomy

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15.1 Introduction

Chicken, and birds in general, have evolved to have a large egg cell with abundant quantity of egg yolk or vitellus in the oocyte cytoplasm that responds extraordinarily well to the reproductive physiological and living needs of the species. The main functions of the egg and its coats are to promote oocyte maturation in the ovarian follicle in such a way that the male pronucleus can survive, to block polyspermy, and to promote embryonic development (Okumura 2017; Tanghe et al. 2002). Geometrically, a chicken egg is an asymmetrical prolate ellipsoid that has a cross-section with a circular shape and a longitudinal section as an asymmetrical ellipse (Burley and Vadehra 1989, p. 4; Mao et al. 2007). One pole is slightly flattened (polus obtusus) and the other pole is more pointed (polus acutus) (Figure 15.1). In almost all avian species, the egg is placed in the nest at an angle with its sharp end down due to its asymmetrical shape, decreasing the possibility of egg damage. The blunt end (polus obtusus) is pointed up, and it is where the air chamber resides with an observable increased volume. This is to facilitate gaseous exchange during embryonic development. The air chamber is created during the egg passage through the oviduct. After oviposition, the eggs are considered to have higher hatchability when the position of the sharp end is down and the blunt end up (Mao et al. 2007). Although there is some variation among the size and shape of avian eggs, they are all constituted by similar structures. The ultimately formed chicken egg consists of four main parts: germinal disc, yolk, albumen with chalaza, and the eggshell (Nickel et al. 1977, p. 81; Pollock and Orosz 2002).

15.2 The Germinal Disc

The germinal disc (also known as the blastodisc or if fertilized, the blastoderm) is a circular slightly raised structure of approximately 3–4 mm in diameter located on the surface of the yolk and is white gray in color. It contains the remnant of the oocyte nucleus while the cytoplasm is an extremely thin layer that covers the rest of the surface of the yolk (Nys et al. 2017) (Figure 15.2).

15.3 The Yolk

The yolk (or vitellus) of a chicken egg is the nutrient-rich, yellow-orange part of the egg that serves as the primary source of nutrition for the developing embryo. It is surrounded by a thin membrane called the *vitelline membrane*, which separates it from the egg white. The yolk consists of lipoproteins and phosphoproteins arranged in concentric layers. Due to the difference in the content of protein and lipids, it can be distinguished into alternating yellow and white layers of the yolk although sometimes it is difficult to observe. In addition to the main components, proteins and lipids, the yolk also contains carbohydrates, vitamins, minerals, and water. It is a highly concentrated source of nutrients and energy and provides the developing embryo with everything needed to grow and develop. The innermost central nucleus of the white vitellus is known as the *latebra* and is connected to the germinal disk through the neck of the latebra (Figure 15.3). The latebra is a small, raised bump or knob that can sometimes be found on the surface of a

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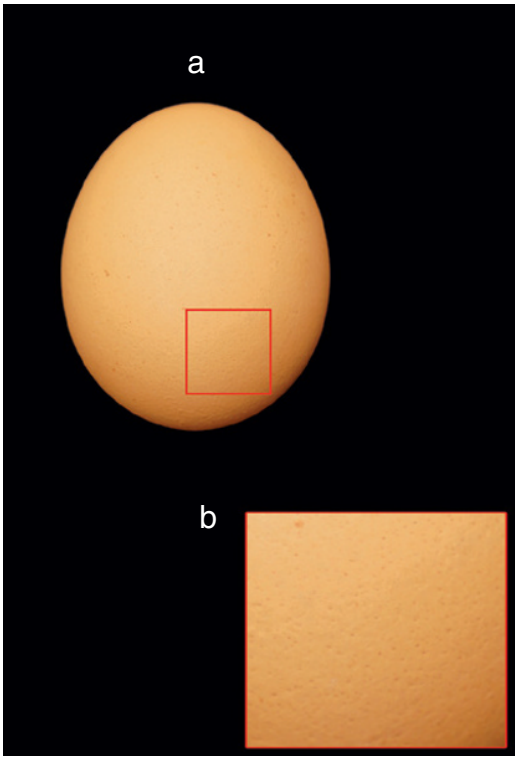

Figure 15.1 Regular brown chicken egg. The pole that is more pointed is the *polus acutus* (a) and the slightly flattened is the *polus obtusus* (b) where the air chamber (cell) is located. At a higher magnification, the eggshell pores can be seen (inset).

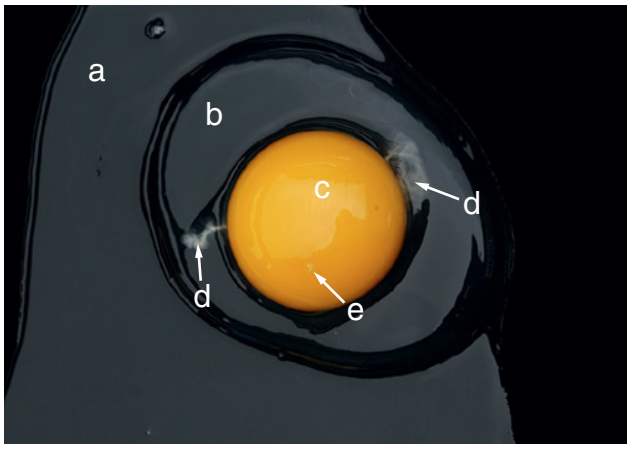

Figure 15.2 The two major compartments of a cracked fresh egg: the albumen (egg white), with the outer thin albumen (a), the inner thick albumen (b), and the yolk (c). Anchored to each pole of the yolk, the chalazae (d) can be observed. On the surface of the yolk, the germinal disc (blastodisc) can be seen as a slightly raised (e), circular structure of approximately 3–4 mm in diameter.

chicken egg yolk. It is located close to the blunt end of the egg, near the air cell, and is formed during the egg formation inside the hen's oviduct. The presence of a latebra on an egg does not affect its quality or nutritional value. The color of the yolk can change depending on the diet of the hen. Thus, diets with a high content of carotenoids (from fruits and vegetables) will produce yolks that are vibrant yellow-orange in color. In contrast, diets low in carotenoid concentration produce yolks that are paler in color.

Proteins constitute a significant portion of the yolk and the most abundant in yolk plasma are serum albumin, ovalbumin, immunoglobulin Y, vitellogenin-derived glycoproteins, and ovotransferrin. The most abundant proteins of the globular fraction of the yolk are apovitellenin-1, vitellogenin-derived lipovitellins, apolipoprotein B-derived fragments, and biotin-binding protein (Mann et al. 2002). Ovalbumin is the most abundant protein in egg whites, but it is also present in small amounts in the yolk. Vitellogenin is a precursor protein that is synthesized in the hen's liver and transported to the developing ova in the ovary. It is then stored in the egg yolk as a source of nutrients for the developing embryo. Vitellogenin is rich in essential amino acids and is an important source of nutrition for the growing chick. Lipovitellin is a phospholipid-containing protein that makes up about 50% of the total protein content of the egg yolk. It is essential for the transport of lipids and other nutrients to the developing embryo. Phosvitin is a phosphorylated protein that is unique to egg yolk. It makes up to 12% of the total protein content of the yolk and is rich in essential amino acids, especially cysteine. Ovotransferrin is a glycoprotein that binds and transports iron in the egg. It also has antibacterial properties and is being studied for potential medical applications. Other proteins found in smaller amounts in egg yolk include lysozyme, ovomucin, ovoinhibitor, and apovitellenin. The proteins in the egg yolk provide a wide range of nutritional and functional properties and are used in many food and non-food applications (Dias et al. 2016).

The other abundant components of the yolk are the lipids, accounting for about 50% of the yolk weight. Egg yolk lipids are primarily composed of triacylglycerols, and phospholipids contained within lipid droplets (Kuksis 1992). The lipids in the yolk provide a concentrated energy source for the developing embryo and play a role in membrane formation and nutrient transport. Carbohydrates are present in smaller amounts in the yolk, with glucose being the most abundant. In lower amounts, fructose, maltose, and sucrose are also found. The function of these carbohydrates is to be the source of energy for the developing embryo. Vitamins and minerals are also present in the yolk and include vitamins A, D, E, and K, as well as calcium, iron, and phosphorus required for the development of the embryo.

The yolk (vitelline) membrane is a clear, elastic, tough coating and the thinnest of all egg layers but could be the principal barrier for fertilization. It forms a protective

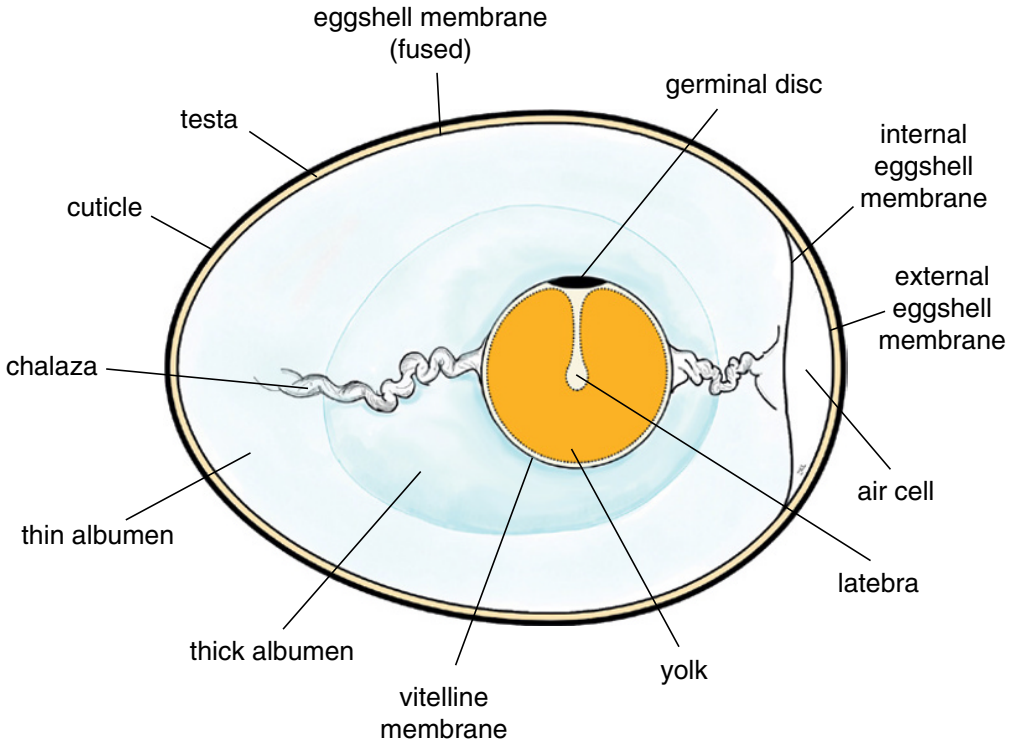

Figure 15.3 A diagram of a hen's egg in a longitudinal section.

barrier between the yolk and the albumen (egg white). This membrane helps protect the yolk from damage and infection and maintains its structural integrity by preventing breaking down or deterioration (Kovacs-Nolan et al. 2005). The vitelline membrane is composed of four different layers that can only be distinguished with electron microscopy. These layers are (a) plasma membrane (plasmalemma) of the oocyte; (b) perivitelline membrane or lamina (also known as the inner layer of vitelline membrane, thin and delicate); (c) continuous membrane or lamina; and (d) extravitelline membrane or lamina (also known as the outer layer of vitelline membrane, thick and fibrous) (Bakst and Howarth 1977). The first two layers are produced by the oocyte and the granulosa cells while still in the ovary as a follicle. The last two layers are produced as the eggs pass through the infundibulum. The yolk membrane is the barrier between the yolk and the albumen but allows for the movement of water, electrolytes, gases, and nutrients (proteins, lipids, and minerals). The exchange of gases such as oxygen and carbon dioxide is essential for the embryo's metabolism.

The yolk is suspended in the center of the egg by the *chalazae*, a white, spring-like structure located at either end of the yolk from the equatorial region of the vitelline membrane into the albumen. It can sometimes be seen when the egg is cracked open (Figures 15.2 and 15.3). It serves as a balancer of the yolk maintaining it in a steady position when the egg is laid (Bellairs and Osmond 2014a,b, p. 5). Since it is a part of the albumen, it will be further explained in the next section.

15.4 The Albumen

The albumen (or egg white) is the main component surrounding the yolk. It is a clear, viscous liquid composed of approximately 90% water and 10% protein, with small amounts of other nutrients such as vitamins and minerals. Although it is described as having less structure than the yolk, two different regions can be identified: a thick (dense) and a thin (liquid) albumen (Figures 15.2 and 15.3), depending on the proportion of water and protein (ovomucin) (King and McLelland 1979, pp. 250–251). The thick albumen is located closest to the yolk and is composed of dense, coiled proteins that help to keep the yolk centered in the egg. The thin albumen is located furthest from the yolk and is composed of more fluid less viscous proteins.

Thick albumen has a higher quantity of ovomucin than thin albumen and consequently has more internal structure. Ovomucin is a glycoprotein that accounts for 3–4% of the total protein content of albumen (Omana et al. 2010). It is a large, complex protein molecule composed of long chains of amino acids, which are linked together by

glycosidic bonds to form a glycoprotein. Ovomucin plays a role in protecting the developing embryo inside the egg. The chalazae (singular: chalaza) are parts of the dense albumen that fix the yolk to the egg poles. They are made of twisted strands of ovomucin fibers arranged in a spiral shape, created by the rotation of the egg while it descends inside the oviduct. As a part of the egg white, the chalaza is also rich in various other proteins, including lysozyme, avidin, and ovotransferrin (Abeyrathne et al. 2014). The lysozyme is an enzyme that helps to break down bacterial cell walls and is an important part of the egg's natural defense against infection. Avidin is a protein that binds to biotin, a vitamin that is important for the metabolism of fats and carbohydrates. This binding helps to prevent biotin absorption by the body. Ovotransferrin is a protein that binds to iron and helps to transport it to developing tissues in the embryo. The chalaza serves several essential functions in the development of the egg, for example, it helps to prevent the yolk from rotating or shifting as the egg transits turning and moving through the female genital tract, which helps to ensure a proper development of the embryo (Roberts 2004).

The thin albumen contains mucins with less fibrous arrangement, and consequently less structural scaffold, giving an appearance of higher fluidity. The inner layer of the thin albumen is attached to the yolk and the outer layer is in contact with the shell membrane, but only at the egg poles.

Besides the ovomucin, other proteins found in the albumen include ovalbumin (54%), ovomucoid (11%), ovotransferrin (12%), and lysozyme (3.5%) (Kato et al. 1986; Sim and Sunwoo 2006; Omana et al. 2010). The albumen also contains a variety of vitamins and minerals, such as riboflavin, niacin, calcium, and potassium. These nutrients are important for the growth and development of the embryo. The quality of the albumen can be influenced by a variety of factors, including the age, diet of the hen, and the storage conditions of the egg. As the egg ages, the albumen becomes thinner and more fluid, and the protein structure begins to break down. These changes can be used to verify the freshness of the egg.

15.5 The Eggshell

The chicken eggshell consists of 95% calcium carbonate and 3.5% organic matter (Mine et al. 2003; Halgrain et al. 2022). This hard, calcareous shell protects the growing embryo from physical trauma, invasion by microorganisms and prevents water loss (dehydration). At the same time, the eggshell allows gaseous exchange while buffering against temperature fluctuations to maintain an appropriate growing temperature for the embryo (Chien et al. 2009).

There are three main parts of what is considered as the eggshell (from inside out): the *shell membranes*, the *testa* or calcified portion, and the *cuticle*.

15.5.1 Shell Membranes

The ***shell membranes*** (membranae testae) are two thin, pliable but strong non-mineralized membranes, composed mainly of several layers of proteinaceous fibers surrounding the albumen (Figure 15.3) (for review, see Arias et al. 1993). They are collectively about 70 μm thick. The inner membrane rests on the surface of the albumen and it is about 20 μm thick and arranged in three layers of fibers composed mainly of collagen (types I, V, and X) and elastin (Arias et al. 1993). The inner shell membrane is fused with the dense albumen only at the egg poles. In the other regions of the egg, the inner shell membrane is in contact with the thin albumen. The more complicated outer membrane has a thickness of 50 μm and is composed of six layers (Gilbert 1979, pp. 239–241). The outer surface of the outer shell membrane is attached to the mammillary knobs from the crystals forming the testa. These knobs indicate the sites at which calcification and formation of the testa begins, each starting as a small center of crystallization on the outer shell membrane. The fibers from the outer shell membrane become encapsulated in the mammillary knobs and it is impossible to separate them without damage (Bellairs and Osmond 2014a,b, p. 5). During the later stages of incubation, the embryo obtains calcium from the eggshell, which leads to a partial demineralization of the testa (Chien et al. 2009). The inner shell membrane is firmly attached to the outer shell membrane, but a few minutes after the egg is laid, and as it cools down, the internal and external membranes detach at the blunt pole creating the *air chamber*. During the process of embryo development, the head of the embryo lies adjacent to this air cell. Egg anatomy assists in egg culture techniques.

15.5.2 Testa

The ***testa*** or calcified portion of the eggshell is mainly composed of two basic parts: the organic matrix (5%) and the inorganic interstitial substance (95%), which is composed of inorganic salts. Both parts are interrelated and intermingled. The organic matrix is the primary biological layer and is composed of a meshwork of fine fibers with different arrangements infiltrated with calcite crystals of the inorganic matrix (Figure 15.4).

15.5.2.1 Inorganic Portion

The inorganic matter represents 90% of the weight of the hen's eggshell and about 98% of this is calcite (calcium carbonate crystals) (Gilbert chapter 5 in King and

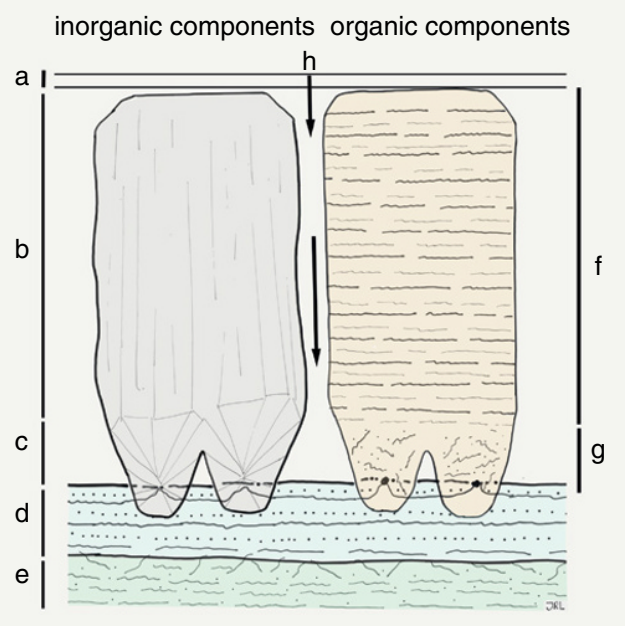

Figure 15.4 A schematic diagram section through the eggshell parts (testa and shell membranes) (King, 1993, p. 389). Cuticle (a). The inorganic components: stratum vallatum (palisade layer) (b), conus mamillae (cone layer) (c), external eggshell membrane (d), and internal eggshell membrane (e). The organic components: stratum spongiosum (f) and stratum mammillarium (g). The pore of the eggshell (h) is covered with a cuticle and leads to the canaliculus testae. *Source:* Adapted from King (1993).

McLelland 1979; Burley and Vadehra 1989; Chien et al. 2009). The remaining 2% of inorganic material is composed of small amounts of magnesium carbonate and tricalcium phosphate. The inorganic material is deposited on top of the organic matrix during the calcification stage of eggshell formation, and it forms a highly ordered crystal lattice structure that provides the eggshell with its characteristic strength and hardness. The calcite crystals are aligned in a specific orientation, which is crucial for the mechanical properties of the shell (Ketta and Tůmová 2016).

The inorganic matrix is composed of two main layers or regions: the cone layer and the palisade layer (stratum vallatum) (Figure 15.4). The *cone layer* is the innermost layer of the testa, and it is composed of small, irregularly shaped calcite crystals that form a rough and porous surface. The *palisade layer* is the outermost layer of the testa, and it is composed of larger, regularly shaped calcite crystals that form a smooth and compact surface. The calcite crystals have a rhombohedral morphology aligned perpendicular to the surface of the outer eggshell membrane, forming a dense and compact layer that is resistant to cracking and fracturing. The deposition of the inorganic matrix occurs through a process called biomineralization, which involves a controlled nucleation and growth of calcite crystals in the presence of the organic matrix. The proteins and glycoproteins in the organic matrix act as templates and modifiers, regulating the size, shape, and orientation of the calcite crystals (Wu et al. 1995; Fernandez et al. 2004).

The inorganic matrix is not a homogeneous material, as it contains various trace elements and impurities that can affect the shell's physical and chemical properties. For example, the presence of magnesium, sodium, and potassium can affect the crystal structure and hardness of the shell, while the presence of copper and zinc can provide antimicrobial properties.

15.5.2.2 Organic Portion

The organic matrix of the eggshell is a mixture of proteins, glycoproteins, and other organic molecules secreted by the eggshell gland during the pre-calcification stage of the eggshell formation. This matrix plays an important role in the formation and structure of the eggshell. The organic matrix is secreted in a series of layers laid down on the egg's surface before calcium carbonate deposits. These layers help to form the shell membranes, which provide a protective barrier against bacterial invasion and mechanical damage. The organic matrix is organized into two layers or regions: the mammillary layer (stratum mamillarium, more internal) and the spongy layer (stratum spongiosum, more external) (Gilbert in King and McLelland 1979; King 1993 in Baumel et al. 1993 N.A. Avium) (Figure 15.4).

The *mammillary layer* represents 20–30% of the thickness of the testa and is characterized by the presence of discrete aggregations of organic matter that intermix with the fibrillar material of the underlying outer shell membranes (Fujii and Tamura 1970). It consists of numerous conical-shaped knobs or mammillae that are suggested to be the sites where the initiation of crystal formation takes place. The proteins in the organic matrix are mostly of the collagen family, which are known for their strength and flexibility. Collagen molecules assemble into fibrils, which then aggregate to form fibers that give the shell its strength and resilience. In the center of each mammilla is a protein core associated with the fibers of the outer shell membrane. During the shell formation, the base of the mammillary cones fuses together to create the scaffold on which the spongy layer is formed (Gilbert in King and McLelland 1979, pp. 251–253). The matrix of the *spongy layer* is composed of fibers that can reach 10 μm in length and 10 nm in diameter with a similar composition to the mammillary fibers. Other non-fiber-like proteins found in the organic matrix are enzymes that regulate the formation and degradation of the eggshell. Some examples of these proteins are ovocleidin-17 (OC-17), involved in the regulation of calcium carbonate crystal formation (Panheleux et al. 2000), ovocleidin-116 (OC-116) with a role in the degradation of the organic matrix during

the process of eggshell resorption (Mann et al. 2002). In addition to proteins and glycoproteins, the organic matrix also contains other organic molecules such as lipids, carbohydrates, and nucleic acids, believed to be involved in the biomineralization process of the eggshell.

The color of the eggshell depends on the breed of chicken and their capacity to deposit pigments like porphyrin and biliverdin precursors and by-products of hemoglobin (Lang and Wells 1987). It is interesting to note that, in general, chickens with red earlobes tend to lay brown eggs and those with white earlobes tend to lay white eggs (Spiegle et al. 2004). Although investigated for some time, these pigments such as biliverdin are synthesized in the shell gland and then deposited onto the eggshell of chickens (Zhao et al. 2006).

In the chicken, the surface of the testa has 7000–17,000 pores (Tyler and Geake 1958). These microscopic pores distributed all over the surface consist of broad, funnel-like openings (10–20 μm in diameter at their inner side and 20–60 μm at the external openings), which open into the pore canals that penetrate the inorganic palisade as single unbranched channels of irregular internal surface and terminate in clefts formed between adjacent mammillary knobs (Parsons 1982) at the level of the outer surface of the outer shell membrane. This passageway from the external surface to the shell membrane is the reason why egg membranes may need to be cultured, as it may provide clues as to the hygienic condition of egg management. These pores are covered and plugged by the cuticle, the outermost organic layer of the egg (Figures 15.1 and 15.4).

15.5.3 Cuticle

The **cuticle** is an extremely thin proteinaceous layer, which plugs the entry to the shell pores and regulates the permeability to gases, liquids, and particles (Board and Halls 1973; Mortola 2009). The cuticle is deposited by the epithelial cells lining the hen uterus during the last 1.5 h before oviposition (Nys et al. 1999). The cuticle thickness on the eggs of domestic hens varies from 0.5 to 12.8 μm and can have an uneven or patchy distribution on the eggshell surface (Board and Halls 1973). Theoretically, the cuticle subserves diverse functions, varying from reducing water loss to the first lines of defense against bacterial penetration by blocking the external surface of pores (Kulshreshtha et al. 2018). It has been shown that cuticle composition is correlated with the hen age and egg freshness, and this information could be useful to design strategies to evaluate and reduce the contamination risks during egg processing (Rodríguez-Navarro et al. 2013). The cuticle consists of 85–87% protein, 3.5–4.4% carbohydrates, 2.5–3.5% lipids, and 3.5% ash (Nys et al. 1999; Rose-Martel et al. 2012; Rodríguez-Navarro et al. 2013). It is recommended to lightly brush off any organic debris from eggs, rather than washing them, to prevent damage to the cuticle. If eggs are to be cleaned then it is recommended to use water that is warmer, specifically 20 °F (11 °C) warmer, than the egg so that contaminants are not drawn into the egg via vacuum action through the pores in the shell (Damerov 1994).

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16

Fertilization and Chick Embryo Development

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16.1 Introduction

In order to identify potential reasons for malformations, lack of development, or embryo mortality during incubation, it is crucial to comprehend the intricate process of normal chicken embryo development inside the egg. Contrary to popular belief that incubation for 21 days is the whole process of development, the success of embryo development relies on essential events that occur before even the egg is laid, including fertilization. These critical early stages of development take place within the oviduct, making them challenging to study, resulting in limited knowledge compared to the later stages, which are directly influenced by these initial processes.

16.2 Avian Fertilization

Fertilization is an indispensable reproductive event to produce the next generation of animals that reproduce sexually. Compared to mammals, avian sperm requires fewer steps to acquire their fertilizing ability. They develop their fertilizing competence while in the testes, and the fact that roosters don't have accessory sex glands allows for the sperm to be deposited directly into the female genital tract (Wishart and Horrocks 2000).

16.2.1 Sperm Deposition, Storage, and Transport

To achieve fertilization, the sperm must encounter the oocytes at a precise time and location. Since avian have internal fertilization, as mammals, sperm needs to migrate within the female genital tract to reach the site where the oocytes are available for fertilization. However, the timing of ovulation in females does not always synchronize perfectly with male insemination. Avian females store sperm in their reproductive tracts as a mechanism to increase the likelihood of gametes encounters (Sasanami et al. 2013). This is crucial because fertilization must occur immediately after ovulation, within a brief time frame, possibly as short as 15 minutes (Wishart and Horrocks 2000), before the outer layer of the vitelline membrane is formed, blocking or making it difficult for the sperm entry.

In avian species, this sperm storage strategy is performed by tubular invaginations known as sperm storage tubules (SSTs) (a.k.a. spermatic tubules, tubuli spermatici or fossulae spermaticae, or sperm nests) that are present in the oviduct (Schindler et al. 1967; Brillard 1993; Bakst 1998) (see Chapter 7 for more information). SSTs are located in the lamina propria of the mucosal folds in the utero-vaginal junction (UVJ) and the infundibulum, with the primary storage site being the UVJ. These spermatic tubules don't seem to have secretory capability but merely act as mechanical receptacles aimed to release viable sperm over time and enable the fertilization of a clutch of eggs without the need for synchronized copulation. These structures retain and nurture the sperm until ovulation occurs or when the oocytes are transported to the site of fertilization. Additionally, sperm storage organs are believed to play a role in sperm selection within the female reproductive tract. This process of sperm and consequently male selection by the female is believed to enhance the genetic quality of offspring and increase the number of offspring by selecting sperm from different males at different periods (Pryke et al. 2010). Thus, the female reproductive tract serves not only as a path for sperm migration to the site of fertilization but also as a natural mechanism ensuring that eggs are fertilized with preferred sperm at the appropriate time and location.

In avian species, sperm exhibit remarkable longevity, surviving for several weeks inside the female genital tract,

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unlike mammalian sperm, which have a relatively short lifespan of a few days. To increase sperm survivability, SST should **uptake**, **maintain**, and strategically **release** the spermatozoa deposited after breeding.

Sperm **uptake** into the SST primarily occurs in the vagina, which serves as the main sperm selection site in birds. Following natural mating, sperm is deposited into the vagina, and the majority (more than 80%) is expelled from there shortly after mating, with less than 1% entering the SST (Bakst et al. 1994). Each tubule can store up to approximately 100 sperm (Bakst et al. 1994). The precise factors leading to the selection of sperm for transportation within the female reproductive tract remain unclear, although factors such as sperm motility (Froman 2003) and vaginal environment have been proposed. In addition to the spermatic tubules found in the UVJ, SSTs have been described in the upper part of the infundibulum where oocyte is fertilized (Bakst and Vinyard 2002).

Sperm **maintenance** in the SST is associated with the period of fertility in birds. These SSTs act primarily as reservoirs, with sperm aligning themselves in an organized manner, heads positioned toward the tubule's epithelium, and tails extending into the lumen (Schuppin et al. 1984; Ito et al. 2011). Within the SST lumen, sperms are densely packed. The resident sperm inside the SST arranges in a way that agglutinates head-to-head, forming dense packs of cells believed to be the basis for prolonged in vivo storage of spermatozoa, as this style of sperm residency is prevalent among domestic birds. In addition, the immune system may influence sperm survivability in the SST since the sperms are recognized as foreign bodies in the oviduct. In this way, the SST may provide protection, shielding sperm from immune responses.

While in the tubules, spermatozoa are likely to be metabolically inactive, as the concentration of calcium in the tubular fluid appears to inhibit sperm motility (Holm et al. 2000). However, upon exiting the tubules, the sperms become activated. The mechanism of sperm storage and viability is further discussed by Das et al. (2008). The duration of sperm fertility varies among hens, but a common range is 18–21 days (Liu et al. 2008). To prove these facts in vitro, several experiments using UVJ mucosal extracts in in vitro cultures with chicken sperm were performed to study the specific effect of the mucosal lining of the SST over the sperm. The major benefits observed were a reduction in energy expenditure from the sperm that led to an increase in sperm lifespan and an enhancement in motility even after 48 hours following the exposure to UVJ extract. In contrast, sperm cultured without these UVJ extracts experienced a complete loss of motility within five hours (Sasanami et al. 2013).

After the spermatozoa are stored for some time in the SST, they need to be **released** in order to arrive at the site of fertilization. Some reports suggest that sperm release occurs in response to the mechanical pressure exerted by a passing ovum, and it is not actively regulated since no contractile elements associated with the SST have been identified (Van Krey et al. 1967; Mero and Ogasawara 1970). However, more recent reports show the presence of neurons, small ganglia, and F-actin in the UVJ of the turkey oviduct suggesting the involvement of an unknown neural factor in the release process (Freedman et al. 2001).

There is also a debate over the timing of when the sperm is released. Some evidence indicates a slow and continuous release of sperm from the SST throughout the ovulatory cycle while other findings suggest that the release of sperm from the SST is closely timed with ovulation and/or oviposition. To support the latter, some studies using scanning electron microscopy were performed after injecting progesterone. Progesterone causes the SST to shrink, leading to the extrusion of a bundle of sperm tails from the SST into the lumen of the oviduct and squeezing out resident sperm during the ovulatory cycle. These results demonstrate that sperm release from the SST is a regulated event during the ovulatory cycle, with progesterone acting as a crucial sperm-releasing factor in birds (Ito et al. 2011). Without proper regulation of sperm release from the SST, a significant portion of the sperm ascending the oviduct may become trapped by descending eggs, potentially impeding successful fertilization (Sasanami et al. 2013).

The domestic hens, as in many wild birds, are typically inseminated by several males (polygynandrous mating). This offers several interesting reproductive physiological processes aimed to select the best and more fit male with a high genetic lineage. From the male side, there is sperm competition (Birkhead and Montgomerie 2020) and from the female side there exist several different mechanisms for postcopulatory sexual selection, including cryptic female choice (Birkhead and Pizzari 2002), sperm–egg interactions (Birkhead and Pizzari 2002), and differential embryo mortality (Newcomer et al. 1999; Pitnick and Brown 2000).

Sperm competition occurs when the sperms of two or more males are present in the female reproductive tract and must compete for egg fertilization. Three mechanisms were proposed to explain the phenomenon of last male sperm precedence (LMSP) basis of the avian sperm competition events (Birkhead and Montgomerie 2020):

(i) sperm stratification within the SST where first inseminations are in disadvantage, (ii) displacement or destruction of already-stored sperm by incoming sperm from a new insemination, (iii) passive sperm loss during the final insemination, a time when previously stored sperm would have mostly been depleted from the SSTs. According to the

models constructed for these three scenarios, displacement of previously stored sperm emerged as the most probable mechanism of LMSP in birds (Lessells and Birkhead 1990; Birkhead and Montgomerie 2020).

16.2.2 Ovulation (Oviposition)

The majority of avian species exhibit year-round egg-laying, with spring being the peak season for most birds to lay most of their eggs for the year. The frequency of egg-laying varies depending on factors such as bird species, fitness, age, and food availability. Mature domestic fowl (*gallus gallus domesticus*) have the capacity to lay eggs throughout the year. During this period, eggs are laid in batches or clutches, with a rate of approximately one egg per day (about every 26–28 hours) over a period of 4–6 days, followed by a resting period of 1–3 days (refer to Chapter 15. The Egg Anatomy for further details). Domestic hens can produce more than 300 eggs per year.

Egg production is hormonally regulated, with the involvement and control of the circadian rhythm, neuroendocrine system, and ovarian-clock mechanisms to dictate the timings of egg-laying events (Hrabia 2022, pp. 947–948). The sequence of events leading to egg-laying begins with the preovulatory release of luteinizing hormone (LH), also known as ovulatory-inducing hormone (OIH). This is followed by ovulation and, finally, oviposition.

Gonadotropin-releasing hormone (GnRH) is synthesized in various brain regions, including the pallium of the olfactory bulb, preoptic area, hypothalamus, and septal regions of the diencephalon. LH is released by the pituitary gland and, in the presence of a sufficiently mature follicle, induces ovulation within approximately 4–8 hours (Gilbert 1971). Progesterone also plays a role, likely exerting its effects through the hypothalamus and regulating the release of LH by the pituitary. For further details, please refer to Hrabia (2022).

Avian oocytes are remarkably large, and their fertilization occurs within the oviduct while new layers are continually forming around them. This prevents studying the sperm–egg interaction through direct observation as it is performed in mammals where *in vitro* fertilization can be implemented. Due to the significant amount of yolk surrounding the avian ovum, there is a necessity to supplement the ovum cell membrane with a vitelline membrane (or perivitelline membrane). The vitelline membrane is the avian egg envelope homologous to the zona pellucida in mammals. The vitelline membrane is formed by two primary layers (Bellairs et al. 1963): the inner layer (or perivitelline layer), which is secreted by the granulosa cells within the ovarian follicle (Bellairs 1965), and the outer layer, which is produced in the oviduct by the cells of the infundibulum (Okamura and Nishiyama 1978). Fertilization takes place in the infundibulum when the outer layer of the vitelline membrane is being formed, a stage at which retains sufficient porosity to allow sperm to swim through it (Okamura and Nishiyama 1978) or just before the laying down of the outer layer of the vitelline membrane, which happens at the junction of the funnel and tubular region (Bellairs and Osmond 2014, p. 3). During the penetration of the inner layer, each sperm releases hydrolytic enzymes from its acrosome to dissolve the granular material located between the fibers (Wishart and Horrocks 2000) in an event called acrosome reaction (AR).

The outer layer primarily consists of ovomucin, lysozyme, and vitelline membrane outer proteins I and II (Burley and Vadehra 1989). Ovomucin forms a fibrous framework for the outer layer, while lysozyme forms an electrostatic complex with ovomucin, providing bulk and strength.

The inner layer of the vitelline membrane mainly consists of specific zona pellucida glycoproteins called ZPs, with five different types identified in birds (ZP1, ZP2, ZP3, ZP4, and ZPD) (Pan et al. 2001; Sasanami et al. 2003; Sato et al. 2009; Kinoshita et al. 2010; Serizawa et al. 2011; Rodler et al. 2012). ZP proteins bind to form the vitelline membrane during follicular development and play a role in the process of sperm–egg binding. Two major glycoproteins, ZP1 and ZP3, are identified with a key role in fertilization. ZP1 is synthesized in the liver and is transported to the ovary via blood circulation. On the other hand, ZP3 is secreted in the granulosa cells. For a more comprehensive discussion of the vitelline membrane's biochemistry, refer to Wishart and Horrocks (2000) and Ichikawa et al. (2011).

16.2.3 Fertilization

Fertilization comprises sequential steps of species-specific sperm–egg binding, induction of the acrosomal reaction or exocytosis, sperm penetration through the oocyte, and the fusion of gametes (Florman and Ducibella 2006) (Figure 16.1).

After the egg enters the infundibulum, numerous sperms rapidly contact the inner layer of the vitelline membrane and penetrate it, by dissolving the granular material between the fibers using hydrolytic enzymes from the acrosome (Wishart and Horrocks 2000). Various stimuli, such as exposure to hormones, chemicals, or egg envelope components, trigger spermatozoa to release the contents of their acrosomal vesicle located at the tip of their head. This process is known as an **acrosome reaction**. The process by which the acrosomal enzymes are released is through the fusion of the plasma membrane with the outer acrosomal membrane (Florman and Ducibella 2006).

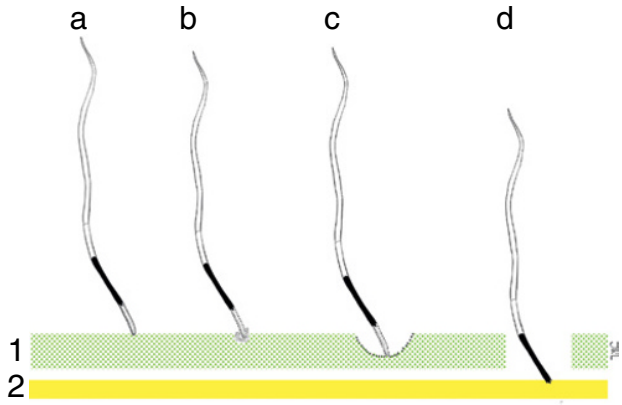

Figure 16.1 Diagram to show the steps for sperm binding to and penetration of the egg perivitelline layer (1) and fusion to the oolemma of the ovum (2). Fertilization comprises sequential steps of sperm–egg binding (a), induction of the acrosomal reaction or exocytosis (b), sperm penetration through the perivitelline layer (c), and the sperm–egg fusion (fusion of gametes) (d).

When bird sperm penetrate the inner layer of the vitelline membrane, they first bind to the carbohydrate-based receptors on it (Robertson et al. 2000). Subsequently, it was discovered that the vitelline membrane possesses AR-inducing activity (Horrocks et al. 2000). AR can be induced when ejaculates are incubated with purified ZP1 or ZPD, another minor constituent of the vitelline membrane in chickens (Okumura et al. 2004). This in vitro AR induction can be inhibited by pertussis toxin (PTX) (Florman and Ducibella 2006). However, the specific components in the vitelline membrane responsible for inducing the authentic AR in any of the avian species are unknown.

Studies using optic, scanning, and transmission electron microscopy have shown that the perivitelline layer of chicken eggs experiences more severe degradation and a higher number of holes near the germinal disc (Bakst and Howarth 1977; Okamura and Nishiyama 1978; Birkhead et al. 1994; Takeuchi et al. 2001).

Following the pass through the vitelline membrane (approximately 30 minutes to 1 hour after ovulation), spermatozoa penetrate deeper, reaching the ooplasm. The inner acrosomal membrane of acrosome-reacted sperm fuses with the vitelline membrane, and the male pronucleus is released, with its sub-acrosomal rod into the egg. Morphological and histological observation using electron microscopy clearly indicates that the plasma membrane fusion occurs between the acrosome-reacted sperm and the egg, in a way similar to that of mammals. During this phase, the sperm nucleus undergoes decondensation and morphological transformation from rod-shaped to pear-shaped (Perry 1987). Concurrently, the male pronucleus begins preparations for fusion with the female pronucleus, located at the center of the germinal disc. At this stage, mitochondria within the sperm midpiece swell and detach (Okamura and Nishiyama 1978).

Throughout most of this period, the female pronucleus remains arrested at the metaphase stage of the second meiotic division and does not complete meiosis or extrude the second polar body until the sperm transformation has reached an advanced stage, which occurs around two to three hours after ovulation (Perry 1987; Wishart and Horrocks 2000).

Multiple spermatozoa appear to penetrate the germinal disc (polyspermy) in birds. Around three hours after ovulation, the paired or fused male and female pronuclei can be observed at the center of the germinal disc, while the extra sperm pronuclei are displaced toward the periphery (Perry 1987). By the fourth hour after ovulation, the zygote nucleus has formed and entered the mitotic phase, leading to the formation of the zygote.

16.3 Chick Embryo Development

Understanding the intricate process of normal chicken development within the egg is crucial for identifying potential causes of embryo malformations, developmental deficiencies, or embryo mortality during incubation. Historically, the chicken embryo was among the first embryos to be extensively studied and described due to the easy accessibility of eggs and the relatively straightforward replication of incubation conditions (Hamburger and Hamilton 1992). By creating a small window in the eggshell and covering it with glass, direct observation of embryo formation became possible. A live chick typically takes 21–22 days to develop (1 day in the oviduct and 20–21 days in the nest or incubator) under normal conditions.

As mentioned before, the initial step in this process occurs in the infundibulum, where the ovum or germinal disk (haploid) is fertilized by the sperm (haploid), resulting in the formation of a zygote (a single diploid cell) (Romanoff 1960; Bellairs and Osmond 2014). The zygote then undergoes a series of cell divisions at the isthmus level and becomes the blastoderm or embryo (Figure 16.2). However, during the laying process, embryonic development is temporarily suspended and resumes upon the temperature increase during natural brooding or artificial incubation. Embryonic activity ceases and development halts when the egg temperature falls below 68 °F (20 °C). Nevertheless, once the temperature reaches 68 °F (20 °C) or higher, embryonic activity recommences.

For maintaining viability, it is crucial to store viable fertilized eggs in cool storage below 68 °F (20 °C) as soon

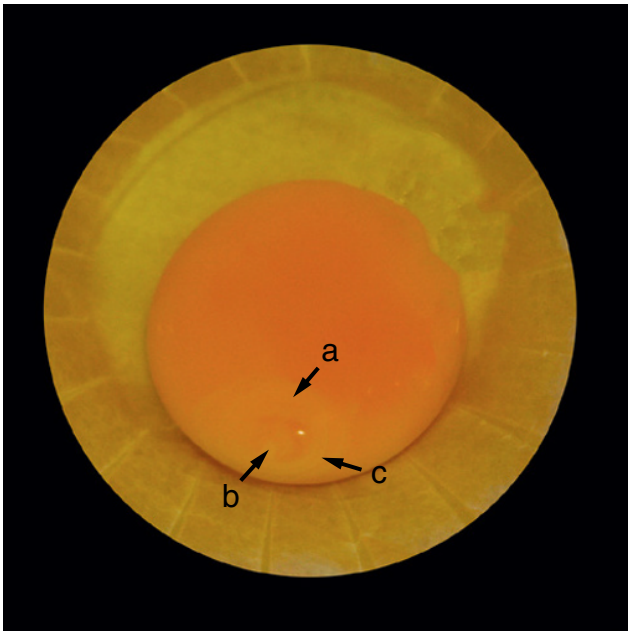

Figure 16.2 Day 2 chicken embryo. (a) Blastoderm as a clear color disc on the yolk surface, (b) area pellucida, specific region where the embryo will develop, no somites are yet visible, and the primitive streak is starting to form at posterior half of embryo, (c) area opaca, extraembryonic annexes at the periphery of the centrally located embryo.

as possible after collection. In the incubator, precise temperature control is essential, with the optimal temperature being 99.5 °F (37.5 °C). Avoiding temperature oscillations is critical for normal embryo development and achieving maximum viability results.

After the zygote is formed and passes through the different segments of the oviduct, it becomes surrounded by the characteristic components of the unfertilized egg (albumen, shell membranes, and testa, see Chapter 15. The egg anatomy). During this period, the embryo undergoes cleavage, which differs significantly from the initial embryo development in mammals. The initial cell divisions (16-cell stage) produce cells that lack complete individualization or a cell membrane. These cells are referred to as "open cells,"where their cytoplasm is exposed to the underlying yolk. At the 64-cell stage, centrally embryonic located cells are entirely surrounded by a cell membrane. However, a region known as the subgerminal and marginal periblast, located beneath and around the embryo, respectively, still contains a mixture of yolk and cytoplasm.

Subsequent mitotic divisions lead to the establishment of axes of polarity. First, the dorso-ventral axis becomes apparent, followed by the cranio-caudal axis. The interaction of these two axes results in the formation of a left and a right side in the embryo and anterior and posterior poles.

The morphological changes during the initial 24 hours can be observed by dissecting the embryo from the yolk's surface and examining it under transmitted light. The most prominent feature is the division into the **area pellucida**, a nearly transparent central region, and the **area opaca**, a more opaque peripheral ring. Sections reveal two layers, the upper layer (**epiblast**), and the lower layer, present throughout the area opaca and posterior part of the area pellucida (Figures 16.2–16.4). The opacity of the area opaca is attributed to the abundance of intracellular yolk droplets in the lower layer. While intracellular yolk droplets are also present in the area pellucida, they are smaller and fewer, rendering the tissue relatively transparent. The area pellucida primarily gives rise to embryonic tissues, while the lateral borders of the area pellucida, along with the entire area opaca, contribute to extraembryonic tissues. The primitive streak becomes visible in the area pellucida around the 6th–7th hour of incubation (stage 2 of Hamburger and Hamilton, 1951). It appears as a dark triangular region in the upper layer, with its apex in the area pellucida and its base along the border with the area opaca. By approximately 18 hours of incubation (stage 2 of Hamburger and Hamilton), the primitive streak reaches its full length, extending about two-thirds of the way across the area pellucida, with Hensen's node visible as the swollen tip at the anterior end. The posterior marginal zone and Koller's sickle are key structures involved in the formation of the primitive streak (Bellairs and Osmond 2014).

Subsequently, the **hypoblast**, the second component of the lower layer, emerges at the posterior end of the area pellucida, originating from cells of Koller's sickle. Approximately 12 hours after incubation, a complete layer called the hypoblast forms beneath the area pellucida. The cells constituting the hypoblast measure 15–20 μm in diameter and are loosely attached to each other and without a basal lamina. The hypoblast serves as the precursor for the endoderm of both the yolk sac stalk and the developing gut. After 24 hours of incubation, the initial blood islands, which serve as precursors to the circulatory system (comprising vessels and cells), become visible on the surface of the yolk sac (extraembryonic mesoderm). This process initiates with the merging of blood islands in the splanchnic mesoderm of the proximal part of the area opaca with those in the distal part of the area pellucida, leading to the formation of a network of capillaries. The development of these simple capillaries occurs through two main mechanisms: vasculogenesis and angiogenesis. These extraembryonic blood vessels play a crucial role in providing nourishment to the developing embryo by facilitating the transport of nutrients from the surrounding extraembryonic membranes. Consequently, the first vessels to emerge are the vitelline vessels, situated atop the yolk sac

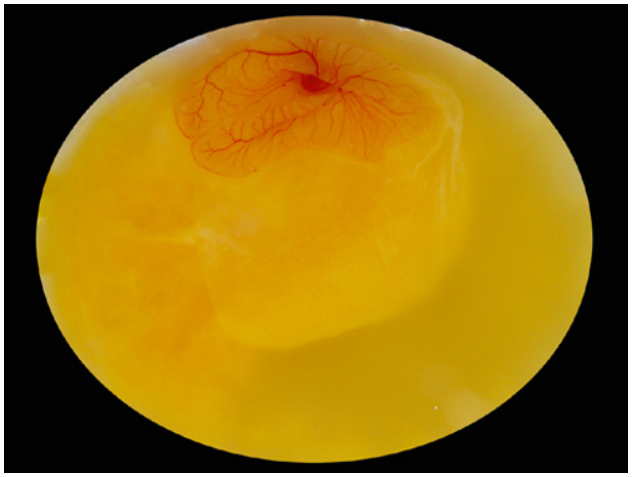

Figure 16.3 Day 3 chicken embryo. Development of extraembryonic circulation converging toward the embryo. The vitelline membrane with vessels expands and covers the yolk surface to enhance the nutrition of the embryo.

(Figure 16.3). Initially, these small vessels consist of a single-layered endothelium. In the chick embryo, the heart tube starts pulsating at 33–38 hours of incubation, with blood flow commencing at 45–49 hours.

Gastrulation is a crucial developmental phase characterized by extensive cell movements, facilitating their correct placement for the subsequent formation of organs and tissues (Bellairs and Osmond 2014). Initially, the epiblastic cells align along the primitive streak before undergoing ingression (Figure 16.4). Ingression involves the departure of cells from the epiblast to integrate into the primitive streak, from which they subsequently migrate to give rise to the definitive endoderm and mesoderm.

During this process, the cells undergo a transformation from an epithelial to a mesenchymal state as they enter the primitive streak (Figure 16.5). Once inside, the cells reorganize into epithelial arrangements again while differentiating into specific tissues. Now, the three germinal layers are formed, which include

1) **Ectoderm**: Responsible for the formation of the nervous system, epidermis, and its derivatives (feathers, beak, claws), along with some skeletal and connective tissues in the head.
2) **Mesoderm**: With specific regions such as paraxial mesoderm (somites responsible for bone and skeletal muscle formation), intermediate mesoderm (responsible for reproductive, urinary, and circulatory systems, including the heart and blood vessels), and lateral mesoderm (forming the lining of the coelom and contributing to the extraembryonic membranes). The head formation and the notochord have also mesenchymal origin.
3) **Endoderm**: Contributes to the lining of the digestive tract and associated organs such as the liver, pancreas, and respiratory system.

During chick development, four associated membranes play essential roles in accessing nutrients from the egg and supporting vital functions (respiration, excretion, and mechanical protection) outside of the hen's body (Figure 16.6). These membranes and their primary functions are as follows:

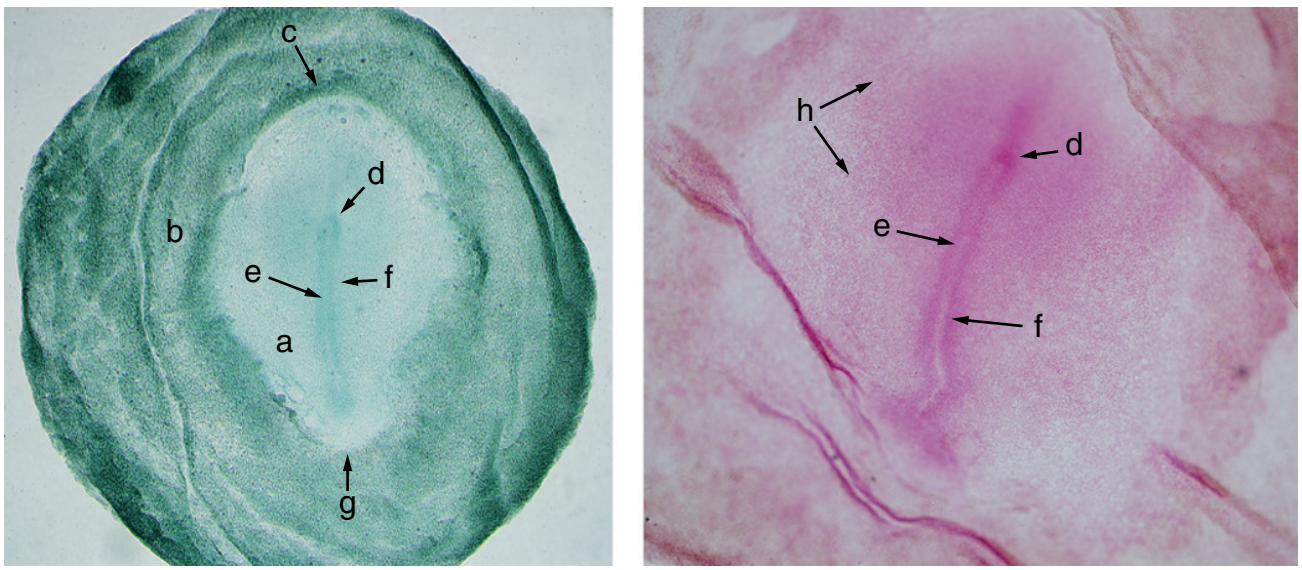

Figure 16.4 Dorsal view of whole mount embryos (18 hours of development). (a) Area pellucida, (b) area opaca, (c) germinal crescent (anterior), (d) Hensen's node, (e) primitive groove, (f) primitive fold (of the primitive streak), (g) Koller's sickle, and (h) limit of migration of the mesoderm.

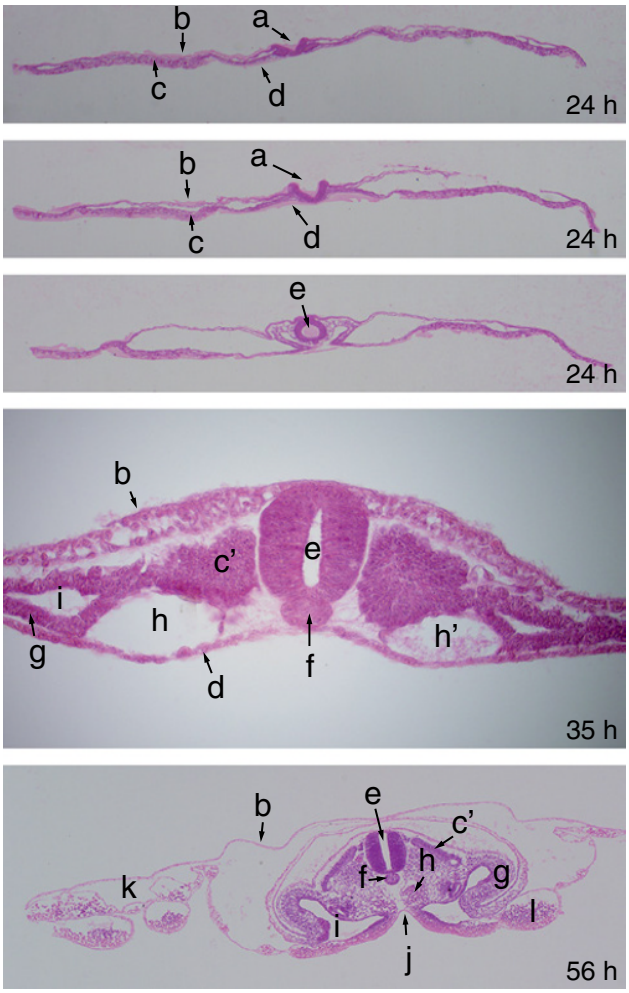

Figure 16.5 Light micrographs of transverse sections from embryos at different stages (24–56 hours of development). (a) Neural plate (open neural tube), (b) ectoderm, (c) mesoderm, (c') lateral masses of mesoderm (somites), (d) endoderm, (e) neural tube, (f) notochord, (g) lateral plate of mesoderm, (h, h') right and left dorsal aorta, (i) embryonic coelom, (j) endoderm of midgut, (k) extraembryonic vessels, and (l) vitelline vein.

1) **Yolk sac:** Initially, the embryo is flat, but it eventually folds cranio-caudally and laterally, creating the yolk stalk. The yolk sac is attached to the embryo at the yolk stalk and primarily serves as a source of nourishment. Around Day 6 of incubation, the yolk sac encloses the entire yolk, facilitating primary vessel growth, blood cell formation, and germ cell differentiation before these cells migrate into their respective organs. By Day 19 of incubation, the yolk sac is drawn into the abdominal/coelomic cavity, although a remnant of the yolk stalk may be found at the Meckel's diverticulum.
2) **Amnion:** The outermost membranes (future chorion) grow dorsally as two separate folds (cranial and caudal). As the embryo sinks, these folds join dorsally, covering and enveloping the embryo to form the amnion and the amniotic cavity. The amniotic cavity is filled with watery fluid (amniotic fluid) that provides buoyancy and protection to the developing embryo from external forces.
3) **Allantois:** The allantois originates during Day 3 of incubation as an outgrowth of the hindgut, extending through the umbilicus near the yolk stalk. It rapidly expands and occupies the space between the amnion and the chorion by Day 10 of incubation. Vessels from and to the heart (umbilical or allantoic arteries and veins) swiftly colonize this membrane, allowing for gas and waste exchange. The allantoic sac plays a crucial role in collecting urinary and digestive waste, particularly uric acid components, which do not diffuse across the allantoic membrane.
4) **Chorion:** The chorion is the outermost membrane that fuses externally with the inner shell membrane and internally with the allantois. The chorion, along with the allantois (forming the chorioallantoic membrane), facilitates gas and water exchange.

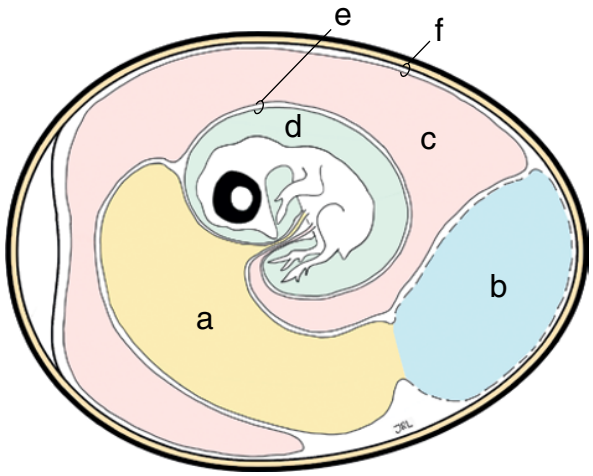

Figure 16.6 Diagram of a chick embryo in a longitudinal section showing the different membranes and sacs formed around the embryo for protection, nourishment, and basic life functions. (a) Yolk sac, (b) albumen sac, (c) allantoic sac, (d) amniotic sac, (e) chorioallantoic membrane, and (f) allantoamniotic membrane.

To determine the developmental stage of chick embryos, a simplified table describing the formation of major organs and structures is provided in Table 16.1 (Hamburger and Hamilton 1992; Warin 2013). Throughout this process, the chick's position progressively changes, with the anterior part of the body facing the larger end of the egg by day 14, the head covered by the right wing by day 18, and the feet in contact with the head during hatching.

Table 16.1 Simplified table describing the developmental stage of chick embryos and the formation of major organs or structures.

Day of incubation	Organs or anatomical structures present
0	Small germinal disc not fertilized
1	Blastoderm, embryonic tissue
2	Formation of blood vessels on top of yolk
3	Leg and wing buds, heart formation, and beats can be observed
4	Formation of eyeball (pigmented area)
5	Identification of limb joints (elbow and knee), digits, and toes
6	Beak and feather tracts
7	Web between toes and egg tooth
8	Initial appearance of feathers, nictitating membrane
9	Phalanges in toes
10	Primordium of comb
11	Allantois has the maximum size, embryo looks like a chick
12	First complete feathers
13	Claws
14	Whole body covered by feathers
15	Vitellus (yolk) shrinks
16	Egg white disappear
17	Urates appear
18	Total growth near completion
19	Yolk sac attached to body cavity
20	No presence or minimal yolk sac
21	Newly hatched chick

Source: Hamburger and Hamilton (1992) and Warin (2013).

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